Metabolic reprogramming and inflammation act in concert to control vascular remodeling in hypoxic pulmonary hypertension

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Stenmark KR, Tuder RM, El Kasmi KC. Metabolic reprogramming and inflammation act in concert to control vascular remodeling in hypoxic pulmonary hypertension. J Appl Physiol 119: 1164–1172, 2015. First published April 30, 2015; doi:10.1152/japplphysiol.00283.2015.—Pulmonary hypertension (PH) is a complex, multifactorial syndrome that remains poorly understood despite decades of research. PH is characterized by profound pulmonary artery (PA) remodeling that includes significant fibro-proliferative and inflammatory changes of the PA adventitia. In line with the emerging concept that PH shares key features with cancer, recent work centers on the idea that PH results from a multistep process driven by reprogramming of gene-expression patterns that govern changes in cell metabolism, inflammation, and proliferation. Data demonstrate that in addition to PA endothelial cells and smooth muscle cells, adventitial fibroblasts from animals with experimental hypoxic PH and from humans with PH (hereafter, termed PH-Fibs) exhibit proinflammatory activation, increased proliferation, and apoptosis resistance, all in the context of metabolic reprogramming to aerobic glycolysis. PH-Fibs can also recruit, retain, and activate naïve macrophages (Mφ) toward a proinflammatory/preremodeling phenotype through secretion of chemokines, cytokines, and glycolytic metabolites, among which IL-6 and lactate play key roles. Furthermore, these fibroblast-activated Mφ (hereafter, termed FAMφ) exhibit aerobic glycolysis together with high expression of arginase 1, Vegfa, and Hif1β, all of which require hypoxia-inducible factor 1α and STAT3 signaling. Strikingly, in situ, the adventitial Mφ phenotype in the remodeled PA closely resembles the Mφ phenotype induced by fibroblasts in vitro (FAMφ), suggesting that FAMφ crosstalk involving metabolic and inflammatory signals is a critical, pathogenetic component of vascular remodeling. This review discusses metabolic and inflammatory changes in fibroblasts and Mφ in PH with the goal of raising ideas about new interventions to abrogate remodeling in hypoxic forms of PH.

glycolysis; HIF; IL-6; fibroblast; macrophage

PEOPLE WITH PULMONARY HYPERTENSION (PH), due to lung diseases and/or hypoxemia, comprise a distinct group (Group 3) in the World Health Organization (WHO) clinical classification system (80). WHO Group 3 comprises a heterogeneous set of diseases sharing the common feature of hypoxia-induced pulmonary vascular remodeling. The presence of PH in these patients, who include survivors of acute lung injury and those with pulmonary fibrosis or chronic obstructive pulmonary disease (COPD), significantly worsens morbidity and mortality. Furthermore, recent data demonstrate that in these patients, PH is markedly underdiagnosed and is specifically associated with substantial mortality (19, 45, 53). Unfortunately, all therapies currently approved for use in PH have been designed for patients with WHO Group 1 PH [so-called pulmonary arterial hypertension (PAH), e.g., idiopathic PAH (iPAH), scleroderma PAH, human immunodeficiency virus-related PAH, etc.], and to date, no clinical trial with these drugs has shown benefit in patients with WHO Group 3 disease (78). Furthermore, whereas oxygen supplementation attenuates PH and improves overall survival in patients with COPD, it remains unclear whether these effects are mediated by improved pulmonary vascular modeling or why some patients with COPD develop severe pulmonary vascular disease despite oxygen therapy (1, 10). Thus the identification of underlying pathogenic mecha-
nisms, which may instruct the development of novel and improved treatment strategies for PH in these patients, is of paramount importance. The goal of this mini-review is to examine evidence supporting the hypothesis that chronic hypoxia can induce adaptations in metabolism of both mesenchymal cells (specifically fibroblasts) and immune cells (macrophages (Mφ)), particularly in genetically susceptible individuals, who induce and maintain signaling networks that drive sustained increases in cell proliferation and inflammatory activation/phenotype (Fig. 1). This cell-proliferation/inflammation nexus is therefore critical to the pathogenesis of PH.

**METABOLIC THEORY OF PH: RELATIONSHIP TO CANCER**

Lung tissue hypoxia, to a variable extent, is common to all of the conditions encountered in patients with Group 3 PH. As the lung cells, including the pulmonary vasculature, react to hypoxia, PH develops; this paradigm is particularly relevant in patients with COPD, sleep-disordered breathing, and fibrosing interstitial lung diseases and those living in altitude (80, 83). Oxygen therapy alone, in these patients, is usually not sufficient to normalize pulmonary arterial pressure, suggesting the involvement of cellular growth mechanisms, which cannot wholly be explained by hypoxic vasoconstriction responses (45). Importantly, in all of these conditions, PH is also consistently associated with early and persistent perivascular inflammation and pulmonary arterial remodeling (87, 93). A striking feature is the predominance of perivascular Mφ surrounding remodeled pulmonary arteries (27, 49, 77). This remodeling involves an imbalance of cell proliferation vs. cell death, which taken in conjunction with inflammation and Mφ activation, has led to the hypothesis that the cellular and molecular features of PH resemble hallmark characteristics of cancer monoclonal behavior (18, 39, 93, 95). It is increasingly recognized that changes in cell metabolism in cancer cells, as well as in cells in the surrounding stroma, including Mφ, are essential for cancer cells to proliferate, migrate, and exhibit proinflammatory characteristics (22, 55, 101). As such, there is an intense effort in the cancer field to define the molecular mechanisms that underlie the coordinated response of cancer cells with their immediate cellular microenvironment, made of cancer-associ-
ated fibroblasts (CAF\textsuperscript{s}) and M\textsuperscript{\phi}. The basis of this interaction relates to changes in metabolism, growth, and inflammation. These pathogenic nodes may offer new opportunities for therapy.

Strikingly, a metabolic adaptation akin to aerobic glycolysis (“Warburg-like”), historically assigned to cancer cells, has also recently been reported in PH (18, 32, 67, 93, 105). These changes have been described to occur in smooth muscle cells (SMCs), endothelial cells, and fibroblasts (34, 67). Additional strong data in support of the importance of this metabolic adaptation are supported by 18-fluorodeoxyglucose (\textsuperscript{\textit{18}}FDG) PET imaging, which has demonstrated increased glucose uptake and metabolism in PAH patients as well as in the monocrotaline rat model of PH (54, 105). Furthermore, \textsuperscript{\textit{18}}FDG uptake and gene-expression studies in pulmonary arterial fibroblasts, isolated from iPAH patients, lend support to the concept that a proliferative and inflammatory pulmonary vascular pathology contributes to the lung \textsuperscript{\textit{18}}FDG PET signal (105). This study also showed that \textsuperscript{\textit{18}}FDG uptake occurs in perivascular mononuclear cells, which accumulate in the adventitial perivascular regions. In vivo studies in the monocrotaline model demonstrated a close correlation between lung \textsuperscript{\textit{18}}FDG uptake and pulmonary vascular remodeling. Importantly, enhancement of oxidative glycolysis with dichloroacetate-mediated inhibition of the enzyme pyruvate dehydrogenase kinase attenuated PH and vascular remodeling in this model and also reduced expression of the glucose transporter GLUT1 typically upregulated in cells exhibiting high glycolysis. These findings correlated with reduced \textsuperscript{\textit{18}}FDG PET signals, which were associated with decreased peripheral vascular muscularization and inflammatory cell accumulation. Collectively, these in vivo and ex vivo observations support a “metabolic hypothesis” for the pathogenesis of PH, whereby a rearrangement of the mitochondrial and cytosolic metabolism, known as the “Warburg effect,” might explain, at least partially, the molecular and functional abnormalities seen in PH cells, including excessive proliferation, apoptosis resistance, and inflammatory activation (67).

**METABOLIC AND INFLAMMATORY CHANGES IN FIBROBLASTS AND M\textsuperscript{\phi} IN CANCER**

Our laboratory has been particularly focused on fibroblasts and M\textsuperscript{\phi} in PH, based on observations of inflammation occurring largely in the perivascular regions in PH (which harbor fibroblasts and M\textsuperscript{\phi}) in humans and animal models of PH (9, 77, 85, 87). Interestingly, similar observations of largely adventitial inflammatory responses, mostly involving M\textsuperscript{\phi}, are observed in systemic circulation in response to changes in blood flow and are necessary for remodeling (89). It seems that despite marked cytokine and chemokine production by medial SMCs, M\textsuperscript{\phi} accumulation occurs largely in the adventitia (48, 108). These observations are consistent with the idea that under most circumstances, the vascular media of almost all arteries appear to be an immune-privileged site (91). Here again, the cancer paradigm is appropriate: consistent with the hypothesis put forth by Dvorak (26), almost 30 years ago—that a “tumor is a wound that never heals”—an ever-increasing body of evidence demonstrates that the fibroblasts and M\textsuperscript{\phi} in tumors, often referred to as CAF\textsuperscript{s} and tumor-associated M\textsuperscript{\phi} (TAM\textsuperscript{s}), are key players in the process of tumorigenesis (39, 62).

Several recent studies have shown that in many cancers, the cancer-promoting and therapy-resistant properties of the tumor stroma largely reside in the activity of fibroblasts and M\textsuperscript{\phi} (61, 62). CAF\textsuperscript{s} have been shown to play roles in many aspects of tumor initiation, progression, and metastasis. CAF\textsuperscript{s} are now implicated in the following: 1) modulating the tumor and microenvironment through the secretion of a large variety of soluble factors; 2) modifying tumor metabolism; 3) remodeling the extracellular matrix of the tumor; 4) regulating cancer stemness; 5) modulating the immune response (and affecting M\textsuperscript{\phi} phenotype); 6) promoting cancer cell migration and metastasis; and 7) altering therapeutic responses in a variety of tumors (12, 13). Of particular relevance to the hypothesis that PH exhibits a critical relationship to cancer is the ability of fibroblasts to secrete cytokines and growth factors, their ability to modify tumor metabolism, and their role in modulating immune responses and M\textsuperscript{\phi} phenotype. A wide variety of growth factors and cytokines has been documented to be produced and released into the tumor microenvironment by fibroblasts (75). The CAF secretome also regulates angiogenesis. VEGF-A as well as CXCL12, CXCL14, and connective tissue growth factor are induced in CAF\textsuperscript{s} by neoplastic cells (4). Interestingly, CAF\textsuperscript{s} also show important metabolic changes that probably promote their own survival as well as promote tumorigenesis. CAF\textsuperscript{s} demonstrate increased autophagy in concert with upregulation of members of the glycolytic pathway, including phosphoglycerate kinase 1 (PGK1). Overexpression of PGK1 in normal fibroblasts results in a myofibroblastic phenotype and an ability to promote tumor cell growth (13, 99). Furthermore, in many tumors, CAF\textsuperscript{s} express glycolytic enzymes related to the Warburg effect, such as the M2 isoform of pyruvate kinase (PKM2), as well as lactate dehydrogenase. This metabolic adaptation toward increased aerobic glycolysis in CAF\textsuperscript{s} is hypothesized to generate lactate and ketones, which are secreted into the intracellular space and can act as paracrine oncometabolites that fuel the oxidative mitochondrial metabolism in neoplastic cells. This phenomenon is often referred to as the “reverse” Warburg effect (68). In addition, lactate and ketones have been recognized to modulate M\textsuperscript{\phi} activation (16).

As noted, chronic inflammation is a prominent risk factor for many cancers. An inflammatory environment, especially in the context of the metabolic environment described above, is known to promote error-prone, high-rate proliferation, thereby facilitating tumorigenesis (37). CAF\textsuperscript{s} mediate tumor-enhancing inflammation by expressing a proinflammatory gene signature, which creates a microenvironment that attracts myeloid cells and supports tumor growth and angiogenesis (92). Importantly, carcinoma cells can induce normal surrounding fibroblasts to turn on a proinflammatory gene signature. Furthermore, accumulating evidence shows that the tumor stroma and CAF\textsuperscript{s}, in particular, actively participate in modulating the immune response to help neoplastic cells escape detection, thereby supporting tumor progression (43). Recent studies have shown that depletion of fibroblast-activating protein \(\alpha\)-positive cells restored immunologic detection and destruction of the tumor, indicating that presumably fibroblasts can act as immunosuppressant cells in the tumor microenvironment (31). Additionally, reports suggest that at least within the pancreas, fibroblast-like cells induce the differentiation of peripheral blood mononuclear cells into immune-suppressive,
myeloid-derived suppressor cells, contributing to T cell inhibition (51). In summary, CAFs possess the ability to manipulate the innate (i.e., \( \Delta \Phi \), natural killer cells) and the adaptive immune system (i.e., T cells) to maintain an aberrant inflammatory environment tailored to promoting tumorigenesis.

**METABOLIC AND INFLAMMATORY CHANGES IN PULMONARY ARTERIAL ADVENTITIAL FIBROBLASTS: PARALLELS TO CAFs**

Fibroblasts are also recognized to play a critical role within the vasculature and its innate immune system in the absence of oncogenic transformation by acting as “sentinel” cells (8, 25, 44, 79, 86, 87), which detect and respond to a variety of local environmental stresses and thereby initiate and coordinate pathophysiological responses (5, 6, 8, 33, 41, 63, 81, 82, 96, 102). We have documented that in both experimental hypoxic PH and human PAH, the pulmonary artery (PA) adventitia harbors activated fibroblasts (hereafter, termed PH-Fibs) with a hyperproliferative, apoptosis-resistant, and proinflammatory phenotype that persists ex vivo over numerous passages in culture (2, 20, 49, 66, 98). This particular phenotype would be centrally involved in cell signaling, shaping the surrounding inflammation-prone microenvironment. Indeed, in line with the cancer paradigm—that stromal cells play a critical role in initiation and perpetuation of inflammation (7, 40, 81, 84, 86, 87, 96, 102)—we have recently shown that PH-Fibs potently recruit, retain, and activate naïve \( \Delta \Phi \) (27, 49). Bovine PH-Fibs exhibit an intriguingly similar cytokine profile to that described for CAFs. We have recently established that these fibroblast-activated \( \Delta \Phi \) (FAM\( \Delta \Phi \)) are polarized to a very distinct, here-tofore undescribed, proinflammatory/protumorphenotyping, in which STAT3 and hypoxia-inducible factor 1α (HIF1α) signaling promote expression of genes implicated in chronic, nonresolving tissue responses in PH [e.g., \( \text{Pim1} \); nuclear factor of activated T cells, cytoplasmic 2; \( \text{Arginase-1} \) (Arg1); \( \text{VEGF} \)] (27). This fibroblast-driven polarization of \( \Delta \Phi \) into a distinct phenotype is similar to that recently described for hypoxic tumor-driven \( \Delta \Phi \) polarization (16, 51). In addition, expression of Arg1, Hif1L, and Vegfa is characteristic of wound-healing \( \Delta \Phi \) (26, 58). Thus \( \Delta \Phi \) activation and expression of HIF1α, Arg1, and Vegfa appear to not only be a functional requirement for \( \Delta \Phi \) to promote physiological wound healing but also can be “high jacked” to shape an environment, not only conducive to tumor growth but also importantly, to promote pathological tissue remodeling. These observations highlight the idea that hypoxia signaling, inflammation, and remodeling and ultimately, PH are linked through stromal cell (fibroblast)/\( \Delta \Phi \) interactions, driven specifically by local changes in a hypoxic or hypoxic-like vascular microenvironment.

Data also show that similar to cancer cells, which reprogram metabolic pathways to support high proliferation (Warburg effect) (3, 23, 24, 57, 71, 88, 93), highly proliferative PH-Fibs exhibit a marked and persistent change in their metabolism toward aerobic glycolysis (i.e., Warburg effect), which is maintained ex vivo (105). However, the mechanisms controlling the metabolic, molecular, and functional changes in the fibroblast phenotype in PH have only recently begun to be investigated. Importantly, observations from our laboratory (49, 66, 87, 93, 95, 98, 106) have documented that cells (including SMCs, endothelial cells, and fibroblasts), derived from the PH vessel wall, exhibit durable ex vivo changes in their metabolic state (aerobic glycolysis), as well as in their proliferative and inflammatory capabilities and in their “molecular phenotype”. Stable, “imprinted” changes in the functional phenotype of cell observations strongly support the idea that these stable changes in cells, especially as they relate to metabolism and cell signaling, could arise from acquired somatic mutations and/or epigenetic change.

Here again, there is a striking parallel to observations in cancer. It is now well accepted that signal transduction regulates metabolism (101). Cancer cells exploit signaling-dependent regulation of metabolism. For example, oncogenic activation of signal transduction pathways drives nutrient uptake and metabolism to support continuous macromolecular biosynthesis and cell proliferation (46, 101). Reciprocally, signaling pathways have been shown to be regulated by metabolism through intracellular nutrient-sensing molecules, such as AMP-activated protein kinase (AMPK) and mammalian target of rapamycin complex 1. In addition, metabolites may serve as indicators of the metabolic status of the cell and through metabolite-sensitive protein modifications, modulate the activity of signaling proteins, metabolic enzymes, and transcriptional regulators (101). Modifications, including acetylation, methylation, glycosylation, and phosphorylation, are all generated from metabolites (56). Acetylation, a protein modification involving the addition of an acetyl group obtained from the metabolite acetyl-CoA, is controlled by the combined activities of acetyltransferases and deacetylases (38). There is evidence that both acetylation and deacetylation can be influenced by nutrient availability, suggesting that protein acetylation could serve as a sensitive indicator of cellular metabolic resources (101). It is also recognized that metabolism itself can be regulated by acetylation. A growing body of evidence indicates that nearly all enzymes involved in glucose and fatty acid metabolism are acetylated (107). For some of these enzymes, such as the critically important PKM2, acetylation seems to modulate their activity in a nutrient-responsive, biologically meaningful way (15, 50, 107). The acetylation status of PKM2 was shown to be relevant to tumor growth, as expression of an acetylation mimic mutant version of PKM2 enhanced the growth of xenograft tumors (50).

Similarities to this concept of reciprocal regulation of metabolism and signaling have been raised in PH. We recently documented that the highly proliferative, apoptotic-resistant, and proinflammatory (high expression of IL-6, CCL2/monocyte chemoattractant protein 1 (MCP-1), CCL12/stromal cell-derived factor 1, IL-1β) phenotype of PH-Fibs, isolated from the pulmonary circulation of humans and calves with severe PH, is mediated, at least in part, by a marked decrease in the expression of microRNA 124 (miR-124) and the increased expression of its direct target, the alternative splicing factor poly (A) polymerase (PTBP1), as well as increased expression of class I histone deacetylases (HDACs), a specific subgroup of the deacetylase mentioned above (98). With the use of miR-124 inhibitors and mimics in human and bovine fibroblasts, we demonstrated that overexpression of miR-124 decreased the proliferation and migration rates of PH-Fibs. Similarly, inhibition of miR-124 (via anti-miR-124) in normal control fibroblasts (CO-Fibs) augmented cell proliferation and migration, demonstrating that miR-124 regulates
proliferative and migratory characteristics of fibroblasts. It should be noted that with the use of both miR array analysis and real-time PCR, we noted decreased expression of several other miRs (including miR-184; miR-21, miR-155) in PH-Fibs (from calves and humans) compared with corresponding controls. We made miR mimics of each of these miRs and transfected them into PH-Fibs, as we did for miR-124. In no case did overexpression of these miRs decrease proliferation, migration, or inflammatory cytokine expression. Collectively, these data suggest that loss of miR-124 in PH-Fibs is a major contributor to their “constitutively” activated phenotype.

To elucidate the mechanisms by which miR-124 regulates cell proliferation and migration, we screened transcript levels of cell cycle-related genes and found that miR-124 positively regulates Notch1, phosphatase and tensin homolog (PTEN), forkhead box O3 (FOXO3), p21/Cip1, and p27/Kip1, all of which were reduced in PH-Fibs. Next, we sought to determine the target of miR-124 upstream of the cell cycle regulator genes, knowing that these genes would have to increase, since miR-124 was decreased, as it suppresses cell cycle genes. Previously published data identified PTBP1 as a direct target of miR-124 and showed that PTBP1 can suppress Notch1 signaling (14, 52). Therefore, we focused on PTBP1, which is an abundantly expressed RNA-binding protein involved in several post-transcriptional regulation events, including repression of RNA alternative-splicing events, activation of internal ribosomal entry site-driven translation, and RNA localization and stability. It is overexpressed in a variety of cancer cells in response to oncogenes, such as cMyC and the transcription factor STAT3. We found that PTBP1 expression is increased in bovine and human PH-Fibs (in vitro), as well as in vivo in PA adventitia of humans and calves with severe PH (98). Overexpression of miR-124 (using miR-124 mimics) in human and bovine PH-Fibs inhibited PTBP1 expression, whereas inhibition of miR-124 (using anti-miR-124) in CO-Fibs upregulated PTBP1 expression. We conducted luciferase/PTBP1 3’ untranslated region assays and proved that PTBP1 is a direct target of miR-124 (98). We also showed that PTBP1 acts upstream of Notch1 and negatively regulates the cell cycle-related genes Notch1, PTEN, FOXO3, p21, and p27. Importantly, it is also known that PTBP1 controls splicing of pyruvate kinase, and its increased expression leads to overexpression of the PKM2 isoform, which as noted above, is an important regulator of the metabolic state of cells. These novel findings demonstrate that miR-124 expression is markedly attenuated in PH-Fibs and results in an increase of an RNA-binding protein, PTBP1, which post-transcriptionally regulates expression of several genes controlling cell proliferation, metabolism, and inflammation.

Given the importance of PTBP1 in regulating the proliferative, metabolic, and inflammatory phenotype of PH-Fibs, we performed experiments elucidating the mechanism controlling expression of miR-124. Since it has previously been shown that miR-124 itself is subject to epigenetic modifications (36, 100), we interrogated whether miR-124 is epigenetically silenced in PH-Fibs. Perhaps not unexpectedly, given the significant change in metabolic status of PH cells (aerobic glycolysis) and the increase in class I HDAC expression, we found that treatment of PH-Fibs with the HDAC inhibitors, suberoylanilide hydroxamic acid and Apidicin, led to a significant increase in miR-124 expression with concurrent decreases in expression of its direct targets, PTBP1 and CCL2/MCP-1 (98). These observations support our previously published data, showing increased class I HDAC activity in cultured PH-Fibs and in hypoxic lungs (11, 106). Furthermore, this suggested that decreased miR-124 expression in PH-Fibs occurred through epigenetic modifications, likely through the removal of acetylation marks on histones, resulting in a more condensed chromatin structure and inhibition of transcription. Such an epigenetic event would explain the constitutively activated phenotype of PH-Fibs, which we have shown to be “reversible” (close to normal) through the application of HDAC inhibitors (11, 106).

In summary, it seems increasingly clear that a fibroblast (fibroblast-like cell) emerges in the adventitia of humans and animals with severe PH that bears a striking resemblance to CAFs. These cells exhibit a Warburg-like glycolytic phenotype and are characterized by excessive proliferation, apoptosis resistance, and a proinflammatory phenotype. Metabolism and signaling pathways in these cells are intimately linked and driven by epigenetic changes that are intimately linked to the metabolic state of the cell (Fig. 1).

**METABOLIC AND INFLAMMATORY CHANGES IN PA FAMΦ**

We propose that the synchronized and mutually interactive signaling between pulmonary perivascular fibroblasts and MΦ is central in PH pulmonary vascular remodeling. There is evidence that MΦ accumulation in the remodeled PA is largely restricted to the adventitia, where MΦ are in close proximity to adventitial fibroblasts and that MΦ critically contribute to the vascular remodeling process (27, 35, 73, 77, 86). Importantly, there is evidence for adventitial MΦ progenitor cells (at least in the systemic circulation in the setting of atherosclerosis) that can serve as a durable pool for adventitial MΦ populations (72). As outlined for fibroblasts above, rapidly increasing evidence supports a role for the Warburg effect in immune activation (65), specifically, in MΦ and dendritic cells (DCs). Initial reports by the Pearce lab (29, 30, 47, 69) have demonstrated that Toll-like receptor-activated DCs undergo a metabolic adaptation from oxidative phosphorylation to aerobic glycolysis and that this metabolic switch is critical for DC maturation and function, while it also regenerates NADP oxidase and citric acid cycle intermediates to support fatty acid production. In DCs, this metabolic switch to aerobic glycolysis is promoted by phosphatidylinositol 3 kinase/Akt signaling and is inhibited by the AMPK, a central regulator of catabolic metabolism that is also displayed by cancer cells (29). More recent reports by the O’Neill lab (64, 90) have similarly shown that increased aerobic glycolysis and reduced oxidative phosphorylation are critical events required for innate MΦ activation in response to LPS. These studies have also identified PKM2 as the critical molecular switch to turn on aerobic glycolysis in LPS-activated MΦ (64). Conversely, the alternatively activated MΦ phenotype (induced by IL-4) depends on increased oxidative phosphorylation and lipolysis (42, 60, 76, 97). It is, however, unresolved which metabolic adaptations occur in and are critical for the functional phenotype of MΦ associated with chronic tissue remodeling and fibrosis, especially because these MΦ often resist categorization into the extremes of M1 (LPS-activated) vs. M2 (IL-4-activated) phenotypes (27); their functional phenotype may be in flux, chang-
ing due to local environmental signaling and metabolic conditions. In addition, in chronic tissue-remodeling conditions, there may be absence of IFN-γ and/or LPS (the M1-defining stimuli) and IL-4/IL-13 (the M2-defining stimuli) but presence of tissue-derived danger signals and cytokines, such as IL-6 (27), which drive MΦ activation toward displaying a considerable overlap between M1 and M2 phenotypic gene-expression markers and thus MΦ function (27); conceivably, these MΦ, including FAMΦ, in vascular remodeling associated with PH, may therefore exhibit metabolic features overlapping with those typically observed in and defining the M1 (LPS-activated) and M2 (IL-4-activated) MΦ phenotypes. Whereas they express HIF1 and features of increased aerobic glycolysis, they also express increased mRNA and protein for Arg1. Importantly, this fibroblast-activated phenotype is critically dependent on STAT3 signaling, which clearly and distinctly differentiates it from M1 and M2 (27).

The major consequence of metabolic adaptation toward aerobic glycolysis in LPS-activated MΦ is generation of increased concentrations of the citric acid cycle intermediate succinate (90), which is a potent prolyl hydroxylase (PHD) inhibitor and thus stabilizer of HIF1. The functional consequence of this LPS-mediated HIF1 stabilization through metabolic reprogramming in MΦ is increased transcriptional activation of the proinflammatory gene Il1b. In addition, in LPS-activated MΦ, increased activity of PKM2 further promotes HIF1-mediated transcriptional induction of IL-1β and other cytokines in colorectal cancer cells (104). PKM2 is also critical for enhancing STAT3 signaling (through increased phosphorylation) (21, 103, 104), whereas STAT3, in turn, increases HIF1 signaling and expression of Glut1, which is important for glucose uptake and maintenance of increased glycolysis, enhancing the anaerobic metabolism (Warburg effect). Thus this PKM2-STAT3-HIF feedforward may be a critical signaling pathway in tissue MΦ associated with areas of remodeling and fibrosis that typically lack strong M1-inducing signals and is not associated with IL-4 and canonical M2 MΦ but instead, exhibits STAT3 and HIF1 signaling (27, 104). Moreover, PKM2 can drive expression of Arg1 through activation of STAT3, which as we have reported, is highly upregulated in FAMΦ (27). Increased activity of Arg1 through metabolism of arginine can have profound effects on restricting arginine availability to “client cells” (e.g., adventitial fibroblasts) (70). Under arginine-limiting conditions, MΦ initiate a salvage pathway (74) that consumes aspartate and generates fumarate, thus directly affecting concentrations of citric acid cycle intermediates that are capable of inhibiting PHDs and thus stabilizing HIF1. Moreover, increased aerobic glycolysis produces increased amounts of lactate. Importantly, because FAMΦ does display evidence of both aerobic glycolysis and citric acid cycle activity (unpublished observations), akin to activated T cells (59), pyruvate is excreted as lactate but also entered into the citric acid cycle; in this regard, lactate has been shown to be a potent activator of MΦ toward expression of HIF1 and Arg1 (16). Together, these metabolic pathways make these cells glutamine-dependent, whereas the arginine metabolism through Arg1 may provide glutamate and help replenish the citric acid cycle (unpublished observations).

As such, activation of STAT3, HIF1, and PKM2 in the molecular crosstalk between MΦ and fibroblasts through bidirectional generation and consumption of metabolites downstream and upstream of aerobic glycolysis may perpetuate rather than attenuate inflammatory activation (Fig. 1).

Intriguingly, inhibition of this PKM2 activity has been shown to convert the LPS-activated phenotype toward a MΦ with anti-inflammatory features, as indicated by increased expression of IL-10 (64). Interestingly, in DCs, IL-10 antagonizes DC maturation through inhibition of metabolic adaptation to aerobic glycolysis (47). Thus MΦ phenotypic conversion toward generation of anti-inflammatory mediators and simultaneously increasing responsiveness of inflammatory MΦ to anti-inflammatory IL-10 might be achieved by interfering with PKM2 activity. However, the ability of IL-10 to inhibit LPS-induced IL-1β transcription in MΦ depends, to some degree, on Arg1, a PKM2-STAT3 target gene (unpublished observations). Precisely how PKM2, HIF1, and aerobic glycolysis affect IL-10 production and IL-10 responsiveness requires further analysis.

The aggregate of these findings demonstrates that functional MΦ plasticity depends on discrete metabolic adaptations and suggests that MΦ can be functionally re-educated through manipulating metabolism. However, whether a similar phenotypic conversion/re-education of MΦ from proinflammatory to a less-inflammatory or even reparative MΦ phenotype can be achieved by inhibition of HIF1 alone or by inhibition of aerobic glycolysis alone awaits further studies. Intriguingly, PKM2 might turn out to play a critical role as a “master regulator” in driving and/or fine-tuning MΦ polarization and phenotypic reprogramming/re-education of MΦ away from proinflammatory or proremodeling phenotypes, because it regulates HIF1 activity and glycolysis, and it also affects STAT3 phosphorylation and signaling (21).

In summary, CAFs and TAMs display considerable overlap with the phenotypes described for PH-Fibs and PH-Fib-activated MΦ associated with vascular remodeling in PH. Therefore, similar molecular and metabolic mechanisms may govern fibroblast-MΦ crosstalk in the process of tumorigenesis and vascular remodeling. PKM2 may prove to be a critical driver of the metabolic and functional crosstalk between CAFs and TAMs, as well as between PH-Fibs and FAMΦ. Thus PKM2 targeting may turn out to attenuate tumorigenesis and vascular remodeling through similar mechanisms. Interestingly, small molecule activators of PKM2 that enhance tetramerization activity have been shown to compromise the proglycolytic and pro-HIF signaling functions (64), and it is conceivable that these molecules also interfere with the ability of PKM2 to phosphorylate STAT3; they may thus prove to be potent inhibitors of the uncontrolled PKM2-STAT3-HIF feedforward signal but may also increase generation of and responsiveness to endogenous anti-inflammatory mediators, such as IL-10.

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