Metabolic Reprogramming and Inflammation Act in Concert to Control Vascular Remodeling in Hypoxic Pulmonary Hypertension

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ABSTRACT

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 include 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 multi-step 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 pro-inflammatory 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 pro-inflammatory/pro-remodeling phenotype through secretion of chemokines, cytokines, and glycolytic metabolites, among, which IL6 and lactate play key roles. Further, these fibroblast-activated Mϕ (hereafter termed FAMϕ) exhibit aerobic glycolysis together with high expression of Arg1, Vegfa, and Il1b, all of which require HIF1α and STAT3 signaling. Strikingly, in situ, the adventitial Mϕ phenotype in the remodeled PA closely resembles the macrophage phenotype induced by fibroblasts in vitro (FAMϕ), suggesting that fibroblast-macrophage cross-talk involving metabolic and inflammatory signals is a critical pathogenetic component of vascular remodeling. This review discusses metabolic and inflammatory changes in fibroblasts and macrophages in PH with the goal of raising ideas about new interventions to abrogate remodeling in hypoxic forms of PH.
INTRODUCTION

People with pulmonary hypertension 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 pulmonary hypertension (PH) in these patients, which include survivors of acute lung injury and those with pulmonary fibrosis or chronic obstructive pulmonary disease (COPD), significantly worsens morbidity and mortality. Further, recent data demonstrate that in these patients PH is markedly under-diagnosed 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 PAH, e.g. idiopathic PAH, scleroderma PAH, HIV-PAH, etc.) and to date, no clinical trial with these drugs has shown benefit in patients with WHO Group 3 disease (78). Further, while 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, identifying underlying pathogenic mechanisms, 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), particularly in genetically susceptible individuals, which 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 the conditions encountered in patients with Group 3 PH. As the lung cells, including the pulmonary vasculature reacts to hypoxia, PH develops; this paradigm is particularly relevant in patients with COPD, sleep disordered breathing, 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 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 macrophages surrounding remodeled pulmonary arteries (28, 49, 77). This remodeling involves an imbalance of cell proliferation vs. cell death, which, taken in conjunction with inflammation and macrophage 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 macrophages, are essential for cancer cells to proliferate, migrate, and exhibit pro-inflammatory 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 associated fibroblasts and macrophages. 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 in fibroblasts (34, 67). Additional strong data in support of the importance of this metabolic adaptation is supported by $^{18}$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). Further, $^{18}$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 $^{18}$FDG PET signal (105). This study also showed that $^{18}$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 $^{18}$FDG-uptake and pulmonary vascular remodeling. Importantly, enhancement of oxidative glycolysis with dichloroacetate-mediated inhibition of the enzyme pyruvate dehydrogenase kinase attenuated pulmonary hypertension and vascular remodeling in this model and also reduced GLUT1 (glucose importer typically up-regulated in cells adapted to high glycolysis) expression. These findings correlated with reduced $^{18}$FDG PET signals, which was 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 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 Macrophages in Cancer**

Our laboratory has been particularly focused on fibroblasts and macrophages in PH based on observations of inflammation occurring largely in the perivascular regions in PH (which harbor fibroblasts and macrophages) in humans and animal models of PH (9, 77, 85, 87). Interestingly, similar observations of largely adventitial inflammatory responses, largely involving macrophages, are observed in systemic circulation in response to changes in blood flow and which are necessary for remodeling (89). It seems that despite marked cytokine and
chemokine production by medial SMCs, macrophage 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 appears to be an immune privileged site (91). Here again, the cancer paradigm is appropriate: consistent with the hypothesis put forth by Dvorak, almost 30 years ago, that a “tumor is a wound that never heals,” an ever increasing body of evidence demonstrates that the fibroblasts and macrophages in tumors, often referred to as cancer associated fibroblasts (CAFs) and tumor associated macrophages (TAMs) are key players in the process of tumorigenesis (26, 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 macrophages (61, 62). CAFs have been shown to play roles in many aspects of tumor initiation, progression, and metastasis. CAFs are now implicated in: 1) modulation of 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 affect macrophage 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 are the ability of fibroblasts to secrete cytokines and growth factors, their ability to modify tumor metabolism, and their role in modulating immune responses and macrophage phenotype. A wide variety of growth factors and cytokines have 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 CTGF are induced in CAFs by neoplastic cells (4). Interestingly, CAFs also show important metabolic changes that probably promote their own survival as well as promote tumorigenesis. CAFs demonstrate increased autophagy in concert with upregulation of members of the glycolytic pathway including (phosphoglycerate kinase-1 (PGK-1)). Upon overexpression
of PGK1 in normal fibroblasts results in a myofibroblastic phenotype and an ability to promote
tumor cell growth (13, 99). Further, in many tumors, CAFs express glycolytic enzymes related to
the Warburg effect, such as the M2 isoform of pyruvate kinase (PKM2) as well as lactate
dehydrogenase (LDH). This metabolic adaptation toward increased aerobic glycolysis in CAFs is
hypothesized to generate lactate and ketones, which are secreted into the intracellular space and
can act as paracrine oncometabolites, that fuel 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 macrophage activation (17).

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). CAFs mediate tumor-enhancing inflammation by expressing a pro-
inflammatory 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 pro-inflammatory gene signature. Further, accumulating
evidence shows that the tumor stroma, and CAFs 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-α
(FAPα)-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 that 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 both the innate (i.e. macrophages, natural killer (NK) 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 Cancer Associated Fibroblasts

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 adventitia harbors activated fibroblasts (hereafter termed PH-Fibs) with a hyper-proliferative, apoptosis-resistant, and pro-inflammatory phenotype, which 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 macrophages (MØs) (27, 49). Bovine PH-Fibs exhibit an intriguingly similar cytokine profile to that described for CAFs (Table 1). We have recently established that these fibroblast-activated MØs are polarized to a very distinct, heretofore undescribed pro-inflammatory/pro-remodeling phenotype, in which STAT3 and HIF-1α signaling promote expression of genes implicated in chronic non-resolving tissue responses in PH (e.g. Pim1 [PIM-1], NfATc2 [nuclear factor of activated T-cells, cytoplasmic 2], Arg1 [Arginase-1], VEGF [vascular endothelial growth factor]) (27). This fibroblast-driven polarization of macrophages into a distinct phenotype is similar to that recently described for hypoxic tumor-driven MØ polarization (16, 51). In addition, expression of Arg1, Hif1, and Vegfa...
is characteristic of wound healing macrophages (26, 58). Thus, macrophage activation and expression of HIF1α, Arg1, and Vegfa appears to not only be a functional requirement for macrophages to promote physiological wound healing, but can also be “high jacked” to shape an environment not only conducive to tumor growth but 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)/Mφ interactions driven specifically by local changes in a hypoxic or hypoxic-like vascular microenvironment.

Data also show that, similar to cancer cells that reprogram metabolic pathways to support high proliferation (Warburg effect) (3, 23, 24, 57, 71, 88, 94), 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 and other laboratories 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” (49, 66, 87, 93, 95, 98, 106). 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 (mTORC1). 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 acyltransferases 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 M2 isoform of pyruvate kinase (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 acetylation mimetic 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 pro-inflammatory (high expression of IL-6, CCL2/MCP-1, CCL12/SDF-1, IL-1β) phenotype of PH-Fibs, isolated from the pulmonary circulation of both humans and calves with severe PH, is mediated, at least in part, by a marked decrease in the expression of miR-124 and the increased expression of its direct target, the alternative splicing factor poly pyrimidine tract–binding protein 1 (PTBP1), as well as increased expression of Class I histone deacetylases (HDACs) a specific subgroup of the deacetylase mentioned above (98). Using 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 utilizing both miRNA array analysis and real time PCR, we
noted decreased expression of several other miRNA (including miR-184; miR-21, miR-155) in
PH-Fibs (from calves and humans) compared to 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
over expression 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, PTEN, 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
posttranscriptional 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 pulmonary artery 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’UTR 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 (HDAC inhibitors), suberoylanilide hydroxamic
acid (SAHA), 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 bear a striking resemblance to CAFs. These cells exhibit a Warburg-like glycolytic phenotype and are characterized by excessive proliferation, apoptosis resistance, and a pro-inflammatory 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 Pulmonary Artery Fibroblast-Activated Macrophages**

We propose the synchronized and mutually interactive signaling between pulmonary perivascular fibroblasts and macrophages is central in PH pulmonary vascular remodeling. There is evidence that macrophage accumulation in the remodeled pulmonary artery is largely restricted to the adventitia, where macrophages are in close proximity to adventitial fibroblasts, and that macrophages critically contribute to the vascular remodeling process (28, 35, 73, 77, 86). Importantly, there is evidence for adventitial macrophage progenitor cells (at least in the systemic circulation in the setting of atherosclerosis), which can serve as a durable pool for adventitial macrophage populations (72). As outlined for fibroblasts above, rapidly increasing evidence supports a role for the Warburg effect in immune activation (65), specifically in macrophages and dendritic cells (DC). Initial reports by the Pearce lab have demonstrated that TLR activated dendritic cells 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 NADPH and citric acid cycle intermediates to support fatty acid production (29, 30, 47, 69). In DCs this metabolic switch to aerobic glycolysis is promoted by PI3K/Akt signaling and is inhibited by the adenosine monophosphate (AMP)–activated protein kinase
AMPK), a central regulator of catabolic metabolism that is also displayed by cancer cells (29). More recent reports by the O’Neill lab have similarly shown that increased aerobic glycolysis and reduced oxidative phosphorylation is a critical event required for innate macrophage activation in response to LPS (64, 90). These studies have also identified PKM2 as the critical molecular switch to turn on aerobic glycolysis in LPS activated macrophages (64). Conversely, the alternatively activated macrophage phenotype (induced by IL4) 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 macrophages associated with chronic tissue remodeling and fibrosis, especially because these macrophages often resist categorization into the extremes of M1 (lipopolysaccharide LPS activated) vs. M2 (IL4 activated) phenotypes (28); their functional phenotype may by in flux, changing due to local environmental signaling and metabolic conditions. In addition, in chronic tissue remodeling conditions, there may be absence of IFNg and/or LPS (the M1 defining stimuli) and IL4/IL13 (the M2 defining stimuli) but presence of tissue derived danger signals and cytokines, such as IL6 (28) that drive macrophage activation towards displaying a considerable overlap between M1 and M2 phenotypic gene expression markers and thus macrophage function (28); conceivably, these macrophages, including fibroblast activated macrophages 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 (IL4 activated) macrophage phenotypes. While they express HIF1 and features of increased aerobic glycolysis they also express increased mRNA and protein for Arginase1. Importantly, this fibroblast-activated phenotype is critically dependent on STAT3 signaling, which clearly and distinctly differentiates them from M1 and M2 (28).

The major consequence of metabolic adaptation towards aerobic glycolysis in LPS activated macrophages is generation of increased concentrations of the citric acid cycle.
intermediate succinate (90), which are a potent PHD inhibitor and thus stabilizer of HIF1. The
functional consequence of this LPS mediated HIF1 stabilization through metabolic
reprogramming in macrophages is increased transcriptional activation of the pro-inflammatory
gene *Il1b*. In addition, in LPS activated macrophages increased activity of PKM2 further
promotes HIF1 mediated transcriptional induction of IL1b and other cytokines in colorectal
cancer cells (104). PKM2 is also critical for enhancing STAT3 signaling (through increased
phosphorylation; (21, 103, 104), while 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 feed-
forward may be a critical signaling pathway in tissue macrophages associated with areas of
remodeling and fibrosis that typically lack strong M1 inducing signals and which are not
associated with IL4 and canonical M2 macrophages but instead exhibit STAT3 and HIF1
signaling (28, 104). Moreover, PKM2 can drive expression of Arginase1 through activation of
STAT3, which as we have reported is highly upregulated in fibroblast-activated macrophages
(28). Increased activity of Arginase1 through metabolism of arginine can have profound effects
on restricting Arginine availability to “client cells” (e.g. adventitial fibroblasts) (70). Under
arginine limiting conditions macrophages 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 fibroblast-activated
macrophages do 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
macrophages toward expression of HIF1 and Arginase1 (17). Together these metabolic pathways
make these cells glutamine dependent while arginine metabolism through Arginase1 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
macrophages 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 towards a macrophage with anti-inflammatory features, as indicated by
increased expression of IL10 (64). Interestingly, in DCs IL10 antagonizes DC maturation
through inhibition of metabolic adaptation to aerobic glycolysis (47). Thus, macrophage
phenotypic conversion towards generation of anti-inflammatory mediators and simultaneously
increasing responsiveness of inflammatory macrophages to anti-inflammatory IL10 might be
achieved by interfering with PKM2 activity. However, the ability of IL10 to inhibit LPS induced
IL1b transcription in macrophages depends to some degree on Arginase1, a PKM2-STAT3 target
gene (unpublished observations). Precisely how PKM2, HIF1, and aerobic glycolysis affect both
IL10 production and IL10 responsiveness requires further analysis.

The aggregate of these findings demonstrates that functional macrophage plasticity
depends on discrete metabolic adaptions and suggests that macrophages can be functionally re-
educated through manipulating metabolism. However, if a similar phenotypic conversion/re-
education of macrophages from pro-inflammatory to a less inflammatory or even reparative
macrophage 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 macrophage polarization and phenotypic re-
programming/re-education of macrophages away from pro-inflammatory or pro-remodeling
phenotypes because it regulates both HIF1 activity and glycolysis and it also affects STAT3 phosphorylation and signaling (21).

In summary, cancer-associated fibroblasts and tumor associated macrophages display considerable overlap with the phenotypes described for PH-Fibs and PH-Fib activated macrophages associated with vascular remodeling in PH. Therefore, similar molecular and metabolic mechanisms may govern fibroblast macrophage crosstalk in the process of tumorigenesis and vascular remodeling. PKM2 may prove to be a critical driver of the metabolic and functional cross-talk between CAFs and TMAs as well as between PH-Fibs and fibroblast-activated macrophages. 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 pro-glycolytic and pro-Hif signaling functions (64) and it is conceivable that these molecules also interfere with PKM2’s ability to phosphorylate STAT3 and they may thus prove to be potent inhibitors of the uncontrolled PKM2/STAT3/HIF feed-forward signal but may also increase generation of and responsiveness to endogenous anti-inflammatory mediators, such as IL10.

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Fig. 1: In line with the emerging concept that pulmonary hypertension (PH) shares key features with cancer, increasing evidence supports the idea that PH results from a multi-step process driven by reprogramming of genes that govern changes in cell metabolism, inflammation, and proliferation. Pulmonary artery adventitial fibroblasts from animals with experimental hypoxic PH and from humans with PAH exhibit pro-inflammatory activation, increased proliferation, and apoptosis resistance, all in the context of metabolic reprogramming to aerobic glycolysis. These activated adventitial fibroblasts can recruit, retain, and activate macrophages toward a distinct pro-inflammatory/pro-remodeling phenotype through the secretion of chemokines, cytokines, and other metabolites including lactate. This fibroblast activated macrophage also exhibits a switch to aerobic glycolysis, which is regulated through a STAT3-HIF1 signaling axis. Collectively, this fibroblast macrophage signaling unit plays a critical role in the persistent inflammation and fibrosis that characterize certain forms of PH.
**Fibroblast**

- Glucose → PKM2 → Glycolysis
- Fumarate → Succinate
- HDAC → miR124 → PTBP1 → Acetyl-CoA → TCA cycle
- PH-Fib proliferation (↓PTEN)

**Cytokines** (IL6, IL1β, MCP1)
- Lactate
- Glutamine
- Arginine

**Vascular Remodeling**
- Persistent Inflammation
- Matrix Production / Fibrosis
- Endothelial Proliferation / Dysfunction
- Stiffening
- Angiogenesis

**Macrophage**

- Glucose
- PKM2 → Glycolysis
- Glut1
- Fumarate
- Succinate
- HIF1
- pSTAT3
- IL1β, TGFβ, VEGF

**PDGF-β, CXCL12, Lysyl-oxidase**