Pulmonary hypertension (PH) (1) is a recalcitrant disease that is increasingly recognized as a critical contributor of cardiovascular mortality worldwide. While considerable progress has been made in the understanding of pathophysiology of PH, significant knowledge gaps still remain. At the same time, our therapeutic repertoire continues to be painfully limited despite additions of several novel classes of PH medications (1,2). These oft-termed advanced therapies [represented by prostaglandin analogues, endothelin-receptor antagonists, and soluble guanylyl cyclase agonists] often find their use hindered by cost as well as by suboptimal therapeutic response. The latter arises partially owing to their collective mechanistic dependence on vasodilation, which overlaps with older-generation agents such as calcium-channel blockers and phosphodiesterase 4 (PDE4) inhibitors. While further dissecting the same set of fundamental pathways may yield incremental benefits, we may need to take a drastically different angle of approach in order to discover drugs with truly ground-breaking efficacy.

In their study published in 12/2017 issue of Circulation, Zhang et al. provided an elegant example of exploring alternative therapeutic approaches with transformative thinking and techniques (3). Over the years, the field of PH research focused on a few signaling pathways involved in vasoconstriction, a process that primarily takes place within the pulmonary smooth muscle layer with regulatory input from the endothelium. In the process, we often overlooked the third histological layer, tunica adventitia, which also contributes to the pathogenesis of PH by way of perivascular inflammation and fibroproliferative remodeling. Fibroblasts are key mediators of the above processes (4,5). Having previously observed that PH-derived fibroblasts displayed profound proliferative, pro-migratory and pro-inflammatory phenotype (6), Zhang et al. built upon the intriguing premise that these fibroblasts undergo metabolic transformation similar to cancer cells (7,8), sacrificing the ATP-efficient oxidative phosphorylation of the mitochondrial citric acid cycle (TCA cycle) cycle and opting instead for aerobic glycolysis that allows generation of metabolic intermediates to be used as cellular building blocks. This phenomenon of metabolic plasticity would transform fibroblasts in PH patients [pulmonary hypertension fibroblast (PH-fibs)] at the very metabolic level, re-routing them to an uninhibited, pro-proliferative cell fate.

Using previously validated techniques, Zhang et al. isolated and cultured fibroblasts from bovine PH models as well as human tissue samples from PH patients and healthy controls. They focused on pyruvate kinase (PK), the enzyme catalyzing the final step of glycolysis and its two functionally distinct splicing isoforms, pyruvate kinase muscle 1 (PKM1) and pyruvate kinase muscle 2 (PKM2). The group noted that PKM2/PKM1 ratio was significantly increased in PH fibroblasts versus that of the controls. This in vitro finding, previously reported in certain subtypes of cancer, was readily reversed with knockdown of PKM2 with isoform-specific siRNA, which interestingly reversed PH-fibs’ abnormal metabolic signature and also blunted their proliferation in vitro. Polyadenosine tract binding protein 1 (PTBP1) has been shown to regulate PK expression and its role in PH pathogenesis has been postulated. The precise mechanisms involved in the regulation of PK expression by PTBP1 in PH-fibs remain to be elucidated. However, the recent discovery of a new axis, miR-124/PTBP1/PKM, that fuels pulmonary hypertension further supports the potential role of this metabolic axis in PH pathogenesis. The authors propose that targeting this axis may provide a novel therapeutic strategy for the management of PH.

**Editorial:**

*miR-124/PTBP1/PKM, a new axis that fuels pulmonary hypertension*

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an alternative splicing factor previously reported to promote PKM2 generation in certain cancer cells (9,10), was found to play a key role in regulating PKM2/PKM1 (6), as siRNA knockdown of PTBP1 decreased the abnormally high PKM2/PKM1 ratio in PH-fibs at both mRNA and protein level. This normalization of PKM2/PKM1 ratio led to restoration of wild-type metabolomics profile in PH-fibs, a finding also seen with treatment by small-molecule modulators/inhibitors of PKM2, TTEP-46 and shikonin. Interestingly, similar metabolic rescue was reproduced when PH-fibs were treated with a mimic of miR-124, an endogenous microRNA of which PTBP1 is a direct target. miR-124 had previously been shown to be pro-apoptotic in glioblastoma (11) and to promote neuronal differentiation via alternative splicing (12), and its down-regulation was linked with tumor invasiveness in various cancers including gastric, bladder and breast cancer (13-15). Additionally, miR-124 was shown to inhibit proliferation in pulmonary artery smooth muscle cells (16) and endothelial cells (17). Using an array of assays including RT-PCR of native transcripts, PKM2 splicing reporter plasmid, western blot, as well as redox assays including respiratory control ratio and mitochondrial superoxide production, Zhang et al. demonstrated that miR124 exerts direct control over PTBP expression, which in turn regulates PKM2/PKM1 ratio and mitochondrial metabolism. It is the first time that a microRNA is established as a central mediator of metabolic homeostasis in pulmonary fibroblasts and thereby the process of adventitial fibrosis and proliferation (see Figure 1 for a graphic illustration of the pathway).

In this study, the role of non-coding RNA is brought into sharp focus. MicroRNAs (or miRNA) are a well-recognized set of noncoding RNAs ~22 nucleotide in length that regulate a wide range of gene expressions through binding to 3’ untranslated region (3’UTR) of target mRNAs, leading to transcript degradation or translational inhibition. Since its discovery in 1993, miRNA has been broadly implicated in many disease pathogenesis including carcinogenesis, heart failure progression and remodeling (18,19), vascular remodeling (20), and even PH (21-23). However, it was speculative at best on how such knowledge might translate into usable clinical tools. Identification of miR-124 as a repressed target with functional importance in the pathogenesis of PH makes it an ideal candidate as a replacement therapy. Indeed, one published report may give a proof-of-concept glimpse, where a miR-124 mimic co-delivered with a Bcl-2 inhibitor in a micelle-based vehicle demonstrated pro-apoptotic effects in breast cancer (24). On a broader perspective, it could join a growing stream of novel oligonucleotide-based therapies on the market or

**Figure 1** Illustration of the miR-124/PTBP/PKM metabolic axis in development of PH. PTBP1 is usually overexpressed in PH-fibs, leading to increased ratio of PKM2 over PKM1 via alternative splicing. miR-124 binds to the 3’UTR of PTBP1 transcripts and decreases its expression, which restores PKM2/PKM1 ratio and reverses the downstream pro-glycolytic, pro-fibrotic and pro-proliferative effects. PH, pulmonary hypertension; PTBP1, polypyrimidine tract binding protein 1; PH-fibs, pulmonary hypertension fibroblasts; PKM, pyruvate kinase muscle.
undergoing clinic trials. Antisense oligonucleotides (ASOs) are a class of synthetic siRNA/miRNA mimetics using a variable of natural and chemically modified backbones. Remarkably, they are able to achieve gene regulation in a variety of ways, including mRNA transcript degradation [RNase H activation or RNA-induced silencing complex (RISC) recruitment], translational inhibition (ribosome assembly disruption or steric hindrance), splicing alteration, and miRNA sequestration (25,26). In 2013, mipomersen was approved by Food and Drug Administration (FDA) for treatment of homozygous familial hypercholesterolemia, which targets apolipoprotein B100 (Apo-B 100) and directs its mRNA degradation via RNase-H activation. PTBP1, overexpressed in PH-fibs, could similarly serve as a valuable therapeutic target, as its in vitro knockdown by siRNA led to mitochondrial metabolic normalization. More recently, two other drugs received accelerated approval from the FDA: eteplirsen for treatment of Duchenne muscular dystrophy (DMD) via exon skipping, and nusinersen for treatment of spinal muscular dystrophy (SMA) via exon retention. The success of these two agents brings into consideration whether a similar approach could be viable in modulating relative expressions of PKM1 & PKM2, which differ only by the inclusion choice of a single exon (exon 9 vs. 10, respectively). It is conceivable that high-throughput screening can identify ASOs with the right sequence and chemical modifications to promote retention of exon 9 over exon 10 (action in opposite to PTBP), thus restoring PKM1/PKM2 ratio in PH-fibs and slowing/reversing adventitial fibrosis and remodeling in the pulmonary vasculature.

This article by Zhang et al. is significant in several unique ways. First, they zoomed in on a separate cell population (fibroblasts) and were able to replicate their findings in both cells from bovine PH models as well as actual human tissues at the time of lung transplant, thus affirming generalizability of their findings. Second, they viewed the field through a metabolic lens, identifying a key regulatory step in glycolysis (miR-124-PTBP1-PKM axis) that disproportionally impacts a cell’s metabolomics profile during PH development. Third and most importantly, the team demonstrated that single-agent intervention at multiple steps of this pathway each led to measurable corrective effects on mitochondrial and cellular bioenergetics. While the results using PKM2 inhibitor/modulators TEPP-46 and shikonin and using histone deacetylases (HDAC) inhibitors offered prospective for traditional small-molecule-based drug strategy, it is the impressive effects of siRNA and miRNA mimetics that may finally usher RNA-based agents onto the stage of PH therapy.

As much as this study was thought-provoking, it had a number of limitations. For instance, non-bioenergetic aspects of mitochondrial function (e.g., Caspase-mediated apoptosis pathway) may be impacted by alterations in PKM2/PKM1 ratio and warrant further study. Second, while changes in the fibroblast proliferative rate in vitro was measured as the main physiologic readout, in vivo aspects of adventitial remodeling in PH (e.g., changes in extracellular matrix or composition; macrophage recruitment/activation) were not examined. The authors did present in the supplemental data (albeit preliminary) that shikonin treatment of mice subjected to hypoxic insult led to normalization of right ventricular (RV) systolic pressure as well as reduction in proliferative, pro-inflammatory, and pro-remodeling markers on lung tissue histology. It would be very interesting to assay for these physiologic effects with direct genetic modulation of PKM2/PKM1 expression, first by generating conditional knockout or transgenic mouse models and eventually using lung-specific ASO and siRNA-delivery vehicles (e.g., nebulization) as explorative therapy.

In summary, Zhang et al. presented the miR-124/PTBP1/PKM axis as a key pro-glycolytic switch that promotes PH fibroblast proliferation via aerobic glycolysis decoupling and cellular metabolic transformation. The study also provided riveting clues that this pathway plays a central role in PH pathogenesis via adventitial remodeling and could be appropriated at multiple steps, most intriguingly at the level of mRNA expression and alternative splicing with ASO-based therapy. The collective findings from this study point to a paradigm shift in the fundamental understanding of PH and intriguing directions towards unlocking brand-new therapeutic options.

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### Footnote

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