immune cells or from the tumor cells as well. Fourth, although there are clear differences in myeloid cells between patients and healthy control patients, there are some reservations as to the exact definition of these cells. It is largely accepted that the definition of MDSCs requires demonstration of T-cell suppression (10, 13), not shown in this study. The authors solely show expression of suppression markers, which though important, are only correlated to immunosuppressive capacity. This is especially problematic in light of the highly controversial issue regarding the definition of neutrophils versus PMN-MDSCs (11, 12). The data shown do not allow for clearly differentiating between these two cell types.

Despite these limitations, the work of Yamauchi and colleagues is an important advancement and the beginning of “the next step” in understanding the immune response to lung cancer. Focusing on the role and effect of myeloid cells in lung cancer has the potential to assist in developing new diagnostic, prognostic, and possibly even therapeutic tools to combat this disease. ■

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References

1. Giroux Leprieur E, Dumenil C, Julie C, Giraud V, Dumoulin J, Labrune S, et al. Immunotherapy revolutionises non-small-cell lung cancer therapy: results, perspectives and new challenges. *Eur J Cancer* 2017; 78:16–23.
2. Gould AP. The Bradshaw Lecture on cancer: delivered at the Royal College of Surgeons of England on Wednesday, December 7th, 1910. *BMJ* 1910;2:1836–1844.
3. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100: 57–70.
4. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell* 2012;21: 309–322.
5. Sharma P, Allison JP. The future of immune checkpoint therapy. *Science* 2015;348:56–61.
6. Forde PM, Chaft JE, Smith KN, Anagnostou V, Cottrell TR, Hellmann MD, et al. Neoadjuvant PD-1 blockade in resectable lung cancer. *N Engl J Med* 2018;378:1976–1986.
7. Gandhi L, Rodríguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, et al.; KEYNOTE-189 Investigators. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. *N Engl J Med* 2018;378: 2078–2092.
8. Engblom C, Pfirschke C, Pittet MJ. The role of myeloid cells in cancer therapies. *Nat Rev Cancer* 2016;16:447–462.
9. Keskinov AA, Shurin MR. Myeloid regulatory cells in tumor spreading and metastasis. *Immunobiology* 2015;220:236–242.
10. Gabrilovich DI. Myeloid-derived suppressor cells. *Cancer Immunol Res* 2017;5:3–8.
11. Brandau S, Moses K, Lang S. The kinship of neutrophils and granulocytic myeloid-derived suppressor cells in cancer: cousins, siblings or twins? *Semin Cancer Biol* 2013;23: 171–182.
12. Granot Z, Fridlender ZG. Plasticity beyond cancer cells and the “immunosuppressive switch.” *Cancer Res* 2015;75:4441–4445.
13. Parker KH, Beury DW, Ostrand-Rosenberg S. Myeloid-derived suppressor cells: critical cells driving immune suppression in the tumor microenvironment. *Adv Cancer Res* 2015;128: 95–139.
14. Yamauchi Y, Safi S, Blattner C, Rathinasamy A, Umansky L, Juenger S, et al. Circulating and tumor myeloid-derived suppressor cells in resectable non-small cell lung cancer. *Am J Respir Crit Care Med* 2018;198:777–787.

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How Many FOXs Are There on The Road to Pulmonary Hypertension?

The Fox (forkhead box) proteins belong to a large family of evolutionary conserved transcription factors (TFs) in the winged helix/forkhead DNA-binding domain. The Fox family includes more than 55 distinct mammalian members grouped into 19 subfamilies (FoxA–FoxS) according to their sequence homology within the DNA-binding domain. Although all Fox proteins share this unique DNA-binding domain, distinct protein domains apart from conserved DNA-binding domain, expression patterns, and post-translational modifications contribute to the divergent functions of Fox family members. The Fox proteins regulate a wide spectrum of biological processes including cell proliferation, apoptosis, differentiation, and resistance to DNA damage (1). As a consequence, either a loss or gain of Fox function can alter cell fate and promote various human pathologies including cancer and cardiovascular diseases. The best-studied Fox proteins

involved in the above-mentioned pathologies are FoxM, FoxO, and FoxP (1).

Among these, FoxM1 is crucial for G1-S and G2-M cell cycle phase progression and mitotic spindle integrity. FoxM1 is highly expressed in proliferating cells including embryonic tissues, adult tissues that have high proliferation index (2), and all solid tumors (3). In contrast, activation of the FoxO family of proteins (FoxO1, FoxO3A, FoxO4, and FoxO6) is associated with cell cycle arrest and the induction of apoptosis (4), and thus, the function of FoxO proteins is often reduced in proliferating cells.

Accumulating evidence suggests that Fox subfamilies FoxM1 and FoxOs are correlated with various biological processes associated with the development of pulmonary hypertension (PH), such as PH initiation and progression. This editorial highlights the complex regulatory mechanisms of FoxM1 and FoxOs in PH, their critical roles in the pathogenesis of PH, their potential as therapeutic targets, and questions remaining to be addressed concerning these issues.

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Figure 1. Role of Fox (forkhead box) transcription factors in pulmonary hypertension. Numerous stresses can activate pulmonary vascular cells including endothelial cells, fibroblasts, and resident and recruited macrophages. Activated vascular cells produce increased amounts of cytokines, chemokines, growth factors, and vasoconstrictors, leading to increased FoxM1 gene expression in smooth muscle cells via reduced FoxO activity, which is a negative transcriptional regulator of FoxM1 gene expression; reduced levels of miR-204, also a negative regulator of FoxM1 mRNA stability and translation; and open FoxM1 chromatin structure, via phosphorylating histones and epigenetic regulators by signal-activated kinases. Increased FoxM1 levels and activity result in smooth muscle cell proliferation, apoptosis resistance, and genome stability by activating genes involved in cell proliferation, antiapoptosis, and DNA repair. CR = cytokine receptor; CXCL12 = chemokine ligand 12; ET-1 = endothelin-1; ETR = endothelin receptor; GFR = growth factor receptor; IGF-1 = insulin-like growth factor-1; Mφ = macrophage; MIF = macrophage migration inhibitory factor; NBS1 = Nijmegen breakage syndrome 1; PDGF-BB = platelet-derived growth factor-BB; TNF-α = tumor necrosis factor-α.

Pulmonary hypertension (PH) is a deadly disease characterized by vasoconstriction and abnormal remodeling of pulmonary vessels, leading to a progressive increase in pulmonary artery pressure, which culminates in right ventricular failure and premature death (5). Observations across species and animal models have demonstrated that numerous stimuli and pathologic conditions (shear stress, hypoxia, oxidative stress, infection, and others) can cause deregulation of normal cellular processes in the pulmonary vasculature, which culminates in abnormalities in proliferation, differentiation, inflammation, and cell death programs, which all together contribute to the development of PH. The initiation process can involve a variety of growth factor, integrin, cytokine, and other ligands, which initiate different signaling cascades, ultimately converging on a common program targeting the activity of certain TFs. Multiple TFs have been implicated in PH development (6). In this issue of the *Journal*, Dai and colleagues (pp. 788–802) identify another TF, FoxM1, as playing a key role in PH development (7). They demonstrate that FoxM1 is markedly upregulated in the lungs of patients with idiopathic pulmonary arterial hypertension (PAH) and various rodent models of PH. These observations are consistent with another recently published manuscript by Bourgeois and colleagues, also demonstrating that FoxM1 is overexpressed in pulmonary artery smooth muscle cells (SMCs) from patients with idiopathic PAH (8). In elegant genetic disruption studies, the authors found that deletion of FoxM1 in SMCs, but not in endothelial cells, in mice protects from hypoxia or sugen/hypoxia-induced PH. Similarly, there is also ample evidence implicating FoxOs, mainly FoxO1 isoform, in PH. Indeed, targeted depletion of FoxO1 specifically in SMCs, both *in vitro* and *in vivo*, is sufficient to induce pulmonary vascular remodeling and PH and also synergizes with the hypertensive effects of hypoxia, resulting in more severe PH (9) and suggesting a causative role of SMC FoxO1 in PH (9) (Figure 1).

Open Questions and Remaining Challenges

Is FoxM1 a critical integrator of multiple signaling pathways driving PH? Dai and colleagues demonstrate that endothelial-derived factors such as CXCL12, PDGF β , ET-1, and MIF are capable of inducing FoxM1 expression in SMCs and of causing SMC proliferation in a FoxM1-dependent manner (7). In addition to the apparent direct stimulatory effects of cytokines and growth factors on FoxM1 gene transcription are findings demonstrating the epigenetic- and TF-mediated regulation of FoxM1. Raghavan and colleagues demonstrated that hypoxia induces FoxM1 gene expression via HIF-2 α , whereas Bourgeois and colleagues demonstrated miR-204-mediated regulation of FoxM1 in pulmonary artery SMCs (8, 10). Further, it has been shown that growth factors and cytokines, which are activated in patients with PH, lead to activation of AKT or JNKs, which then leads to the phosphorylation and nuclear exclusion of FoxOs, and thus upregulation of FoxM1 (9) (Figure 1). Although this study shows a role for endothelial-derived factors in controlling FoxM1 production, it is clear that other cell–cell interactions in the vessel wall may also regulate SMC FoxM1 expression. Future studies should evaluate the role of FoxM1 in inflammatory cells and fibroblasts, as well as the interactions between these cells and SMCs (Figure 1).

What are the consequences of FoxM1 deregulation? Dai and colleagues showed that FoxM1 upregulation leads to SMC hyperproliferation, and subsequently vascular remodeling. In addition, Bourgeois and colleagues showed that FoxM1 deficiency reduces resistance to apoptosis through diminished DNA repair mechanisms and increased survivin expression (8). Recent studies suggest mitochondrial, metabolic, and inflammatory remodeling as critical pathogenetic components of PH (11). FoxM1 being a target gene of FoxOs (major metabolic regulators among the Fox family members) (12), as well as an interacting partner of various TFs, such as NF κ B, β -catenin, and STAT3, suggests a better understanding of the regulation of FoxM1 in the above-mentioned remodeling processes could provide interesting insights into PH development. Identification of FoxM1-FoxO axis target genes and gene networks in PH vascular cells will be very important.

Is FoxM1 a potential therapeutic target for PH? The studies from both Dai and Bourgeois demonstrate that inhibition of FoxM1 with thiostrepton significantly improved established PH in both hypoxia+SU5416 and monocrotaline-challenged rats. Furthermore, FoxO activators or HDAC (histone deacetylase) inhibitors can indirectly target FoxM1. It may be that the beneficial effects of FoxM1 inhibition *in vivo* are the result of a cumulative effect on numerous signaling pathways. Because of the critical role of FoxM1 in normal cell proliferation, maintenance of genome stability, and DNA damage repair, it will be essential to specifically deliver FoxM1 inhibitors to the pulmonary vasculature to reduce/avoid the potential adverse effect of loss of FoxM1 activity in proliferating normal cells.

Conclusions

FoxM1 is implicated in SMC proliferation, which contributes to pulmonary vascular remodeling and PH. Thus, targeting FoxM1 signaling represents a novel strategy for treatment of PAH. However, a deeper understanding of the upstream and downstream molecular mechanisms mediating FoxM1 control of PH (e.g., FoxM1 regulation and role in different subgroups of PH, the mechanisms driving FoxM1 regulation, and the molecular and cellular effects driven by FoxM1 in PH-vascular cells) is essential for developing tailored therapeutic concepts focusing on FoxM1 inhibition. ■

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References

1. Lam EW, Brosens JJ, Gomes AR, Koo CY. Forkhead box proteins: tuning forks for transcriptional harmony. *Nat Rev Cancer* 2013;13:482–495.
2. Wang Z, Ahmad A, Li Y, Banerjee S, Kong D, Sarkar FH. Forkhead box M1 transcription factor: a novel target for cancer therapy. *Cancer Treat Rev* 2010;36:151–156.
3. Halasi M, Gartel AL. FOX(M1) news—it is cancer. *Mol Cancer Ther* 2013;12:245–254.
4. Eijkelenboom A, Burgering BM. FOXOs: signalling integrators for homeostasis maintenance. *Nat Rev Mol Cell Biol* 2013;14:83–97.
5. Hoeper MM, Bogaard HJ, Condliffe R, Frantz R, Khanna D, Kurzyna M, et al. Definitions and diagnosis of pulmonary hypertension. *J Am Coll Cardiol* 2013; 62(25, Suppl)D42–D50.
6. Pullamsetti SS, Perros F, Chelladurai P, Yuan J, Stenmark K. Transcription factors, transcriptional coregulators, and epigenetic modulation in the control of pulmonary vascular cell phenotype: therapeutic implications for pulmonary hypertension (2015 Grover Conference series). *Pulm Circ* 2016;6:448–464.
7. Dai Z, Zhu MM, Peng Y, Jin H, Machireddy N, Qian Z, et al. Endothelial and smooth muscle cell interaction via FoxM1 signaling mediates vascular remodeling and pulmonary hypertension. *Am J Respir Crit Care Med* 2018;198:788–802.
8. Bourgeois A, Lambert C, Habbout K, Ranchoux B, Paquet-Marceau S, Trinh I, et al. FOXM1 promotes pulmonary artery smooth muscle cell expansion in pulmonary arterial hypertension. *J Mol Med (Berl)* 2018;96:223–235.
9. Savai R, Al-Tamari HM, Sedding D, Kojonazarov B, Muecke C, Teske R, et al. Pro-proliferative and inflammatory signaling converge on FoxO1 transcription factor in pulmonary hypertension. *Nat Med* 2014;20:1289–1300.
10. Raghavan A, Zhou G, Zhou Q, Ibe JC, Ramchandran R, Yang Q, et al. Hypoxia-induced pulmonary arterial smooth muscle cell proliferation is controlled by forkhead box M1. *Am J Respir Cell Mol Biol* 2012;46:431–436.
11. D'Alessandro A, El Kasmi KC, Plecité-Hlavatá L, Ježek P, Li M, Zhang H, et al. Hallmarks of Pulmonary Hypertension: Mesenchymal and Inflammatory Cell Metabolic Reprogramming. *Antioxid Redox Signal* 2018;28:230–250.
12. Sengupta A, Kalinichenko VV, Yutzey KE. FoxO1 and FoxM1 transcription factors have antagonistic functions in neonatal cardiomyocyte cell-cycle withdrawal and IGF1 gene regulation. *Circ Res* 2013;112:267–277.

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Know Your Limitations: Assumptions in the Single-Beat Method for Estimating Right Ventricular–Pulmonary Vascular Coupling

Nearly 45 years ago, Suga and colleagues (1) proposed a new approach for quantifying heart pump function based on the concept of elastance or elasticity. Elasticity is a material property that describes how much stress is required to cause material deformation. To illustrate, if the ventricle is a balloon, its elasticity can be measured by how much air (or blood) pressure is required to cause an increase in volume. Unlike a balloon that has constant material properties, a ventricle has time-changing material properties. During filling, the ventricle has a low elasticity, which enables a large increase in volume for a small increase in pressure; during isovolumic contraction, the elasticity increases such that ventricular pressure increases, even though there is no change in volume. Then, during ejection and isovolumic relaxation, elasticity increases and decreases to its diastolic value. One benefit of the time-varying elasticity, or elastance, is that it can be measured from pressure–volume loops. A second is that it is load independent for the left ventricle (1), as well as the right ventricle (2).

Building on the concept of ventricular time-varying elastance, but turning attention toward the problem of the “interaction between the right heart ventricle and its arterial load” (3), Hroar Piene proposed that ventricular elastance should match or couple to the opposition to pulsatile flow in the pulmonary vasculature when “a linkage exists between their two structures”; that is, during systole. Unfortunately, he could not arrive at an analytical solution because the time-varying opposition to flow, or impedance, was computed from a graphical analysis of pressure and flow waves at the time. A year later, Sunagawa and others showed that matching end-systolic elastance (Ees), measured from the ratio of end-systolic pressure to end-systolic volume over multiple heart beats with varying preload, to arterial elastance at end systole (Ea), calculated as end-systolic pressure divided by stroke volume, was a simple and robust approach for assessing ventricular–vascular or ventriculoarterial interactions (or ventricular–vascular coupling) (4).

This new concept found broader use with the development of estimates of Ees based on a single heartbeat instead of multiple beats with varying preload. This “single-beat” method was first developed by Sunagawa and colleagues and validated for the left ventricle, using data from healthy dogs and humans (5, 6), and then validated for the right ventricle using data from healthy dogs (7).

Fast forward to today, and an informal survey of PubMed using search terms such as (“right ventricle” AND “pulmonary

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