Targeting Chromatin Structure: Combined Inhibition of Histone Deacetylases and Bromodomain Proteins in Normalizing Persistent Activation of Pulmonary Hypertensive Adventitial Fibroblasts

Hui Zhang¹, Aya Laux ², Min Li³, Xue-dong Liu³, Nicholas W. Morrell⁴, Cheng-Jun Hu², Kurt Stenmark¹

¹Cardiovascular Pulmonary Research Laboratories, Departments of Pediatrics and Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA; ²Department of Craniofacial Biology School of Dental Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA; ³Department of Biochemistry, JSCBB, University of Colorado, Boulder, CO, USA; ⁴Division of Respiratory Medicine, Department of Medicine, University of Cambridge School of Clinical Medicine, Addenbrooke's Hospital, Cambridge, UK

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Rationale

- The multifactorial nature of pulmonary hypertension (PH) suggests epigenetic changes as potential determinants of vascular remodeling characterized by dysregulated proliferation, apoptosis resistance, and persistent inflammatory signaling of vascular cells through regulating gene expression (both up-regulated and down-regulated genes).
- Epigenetic modifications play a key role in a cell-type specific gene expression patterns. Changes in the chromatin state (especially histone acetylation) of specific genes can lead to their repression or activation (1).

We and others have demonstrated histone deacetylases (HDACs) and histone acetylation readers (BRDs) are dysregulated in animals and/or humans PH. HDAC inhibitors (HDACi) or BRD inhibitors (BRDi) can prevent and ameliorate pulmonary hypertensive changes in vitro and in vivo by normalizing pathological gene expression (2-4).

However, the non-histone protein targets and the narrow therapeutic window of currently available HDACi limits their clinical utility. Isoform-selective HDACi and dose minimizing are viable approaches to circumvent off-target effects.

In this study, to further explore the therapeutic potential of epigenetic targeted mechanisms in PH, we evaluated the effect of combination treatments using the HDACi (targeting decreased genes) together with BRDi (targeting increased genes), in low doses. A new HDACi (OKI-005) with high potency and selectivity for HDACs was used. OKI-005 (US patent) demonstrates potent inhibition of the Class 1 HDACs with far less toxicity than current HDACs, for example, Romidepsin (5-6).

Cultured human pulmonary artery fibroblasts derived from patients with IPAH (PH-Fibs) or from control donors (CO-Fibs) were used as in vitro model system.

Hypothesis

Combination of HDACi that selectively increase pathologically repressed genes, and epigenetic inhibitors such as BRDi that specifically reduce over-expressed genes, both at low concentrations, could be effective in PH treatment with manageable side effects.

Results

1. PH-Fibs Exhibit Increased Expression of BRD2, BRD4 and P300/CBP- associated factor (PCAF) compared to CO-Fibs

2. Increased expression of CXCL2 (SDF1) in PH-Fibs correlates with opened chromatin structure and BRD4, and BRDs (JQ1) can decrease expression in a general dose dependent trend (same trend observed in other increased genes, such as CCL2)

3. At the low doses, JQ1 (100 nM) alone is sufficient to normalize most of increased genes, while OKI-005 was more effective in normalizing decreased genes in PH-Fibs. Proliferation marker (MK67) and anti-apoptotic genes (BCL2) were most altered by JQ1 100 nM combined with OKI-005 64 nM.

4. We found the treatment with low doses of HDACi and BRD alone or in combination significantly decreased PH-Fibs proliferation, and the combination JQ1 and OKI-005 was more effective in inhibiting PH-Fibs proliferation without significantly affecting CO-Fibs (CyQUANT).

Summary

- BRDi exhibited the most significant effect on increased genes in a dose dependent manner, while HDACi was more effective in normalizing decreased genes in PH-Fibs.
- The combination of low dose of JQ1 (100 nM) and OKI-005 (64 nM) was sufficient to normalize both increased genes and decreased genes thus inhibiting the persistently activated PH-phenotype.
- We also found that this combination regimen had significant therapeutic effects on PH-Fibs and had no deleterious effects on CO-Fibs (e.g., proliferation).

Conclusion

- Combination of HDACi and BRDi in low doses is effective in normalizing the persistent activation of PH-Fibs without deleterious effects on control cells, offering promise for epigenetic-targeted therapies in PH.

References

5. ESZey, P. et al. Dysregulated Histone Deacetylases as a novel, class I specific histone deacetylase inhibitor in phase 1 clinical trial. JBC 2018; 293(50):18115-18127.