MG149 inhibits agonist-induced contractile signaling in human airway smooth muscle (HASM) cells

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Abstract

RATIONALE: Asthma is a disease characterized by inflammation, reversible airflow obstruction, and increased airway hyperresponsiveness. Inhaled corticosteroids are a mainstay treatment for asthma, with a proportion of the severe asthma population becoming refractory to this treatment. Mechanisms underlying how corticosteroids modulate the contractile status of airways remain largely unknown. Previously, we found that steroid treatment of HASM resulted in alterations in histone acetylation and gene expression, potentially modulating contractile gene expression. Given these data, we hypothesized that inhibition of the histone acetyltransferase males absent on the first (MOF) will modulate contractile signaling in HASM.

METHODS: Primary HASM cells were grown to confluence, serum starved, and treated with MG149 (1-100 µM, 24 hr) or GSK126 (0.1-30 µM, 24hr). Cells were then stimulated with Carbachol (Cch, 20 µM, 10 min), Histamine (Hist, 1 µM, 10 min), Isoproterenol (Iso, 1 µM, 5 min), or combinations. Cell lysates were prepared and immunoblotted for phosphorylated and total myosin light chain (pMLC/MLC), phosphorylated and total Akt (pAkt/Akt), phosphorylated and total myosin light chain phosphatase (pMYPT1/MYPT1), and enhancer of zeste homolog 2 (EZH2).

RESULTS: MG149 pretreatment attenuated agonist-induced phosphorylation of MLC, Akt, and MYPT1. Interestingly, MG149 treatment also diminished expression of EZH2 in some, but not all, HASM cell lines.

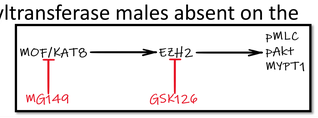
CONCLUSIONS: Our data demonstrate that inhibition of MOF modulates agonist-induced contractile signaling, potentially through modulation of EZH2 expression in HASM. These data suggest that modulation of histone acetylation may be an interesting therapeutic target to attenuate the airway. hyperresponsiveness that is characteristic of asthma.

Background

- Hypercontractility in human airway smooth muscle cells is a characteristic of asthma.
- MYPT1, MLC, and Akt proteins are contractile endpoints in pathways that promote hypercontractility of HASM.
- KAT8/MOF (males absent on the first) is a histone acetyltransferase that modulates acetylation of H4K16. [2]
- EZH2 (enhancer of zeste homolog 2) is a methyltransferase and component of polycomb repressive complex 2 (PRC2) that controls gene expression via methylating histone H3 at lysine 27 (H3K27). [1]
- EZH2 modulates the activity of MYPT1, MLC, and Akt.
- Studies in human oral tongue squamous cell carcinomas suggested that hMOF modulates the activity of EZH2. [2]
- MG149 is a known 6-alkylsalicylate histone acetyltransferase inhibitor of MOF. [2]
- GSK126 is an EZH2-selective inhibitor. [4]
- Carbachol and Histamine are contractile agonists, while Isoproterenol is a contractile antagonist.

Hypothesis

Inhibition of the histone acetyltransferase males absent on the first (MOF) will modulate contractile signaling in HASM through EZH2 modulation.



Methods

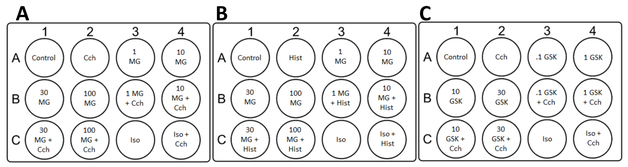


Figure 1. HASM cells from donors were treated in 12-well plates based on the following layouts. (**A**) Cells in this experiment were treated with 1-100 µM MG149 for 24 hr, stimulated with 20 µM Carbachol (Cch) for 10 min, and/or stimulated with 1 µM Isoproterenol (Iso) for 5 min. (**B**) Cells in this experiment were treated with 1-100 µM MG149 for 24 hr, stimulated with 1 µM Histamine (Hist) for 10 min, and/or stimulated with 1 µM Isoproterenol (Iso) for 5 min. (**C**) Cells in this experiment were treated with 0.1-30 µM GSK126 for 24 hr, stimulated with 20 µM Carbachol (Cch) for 10 min, and/or stimulated with 1 µM Isoproterenol (Iso) for 5 min.

EZH2 expression is decreased with inhibition of hMOF

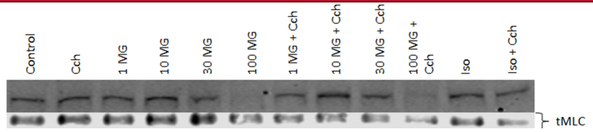


Figure 2. EZH2 and MLC derived from N100217 donor HASM were assessed using Western blots, and a dose-dependent response to 10-100µM MG149 alone and 10-100µM MG149 with Carbachol (Cch) stimulation was observed with decreasing EZH2 expression. This pattern is representative of the group mean. A similar dose-dependent response was observed with the use of Histamine as the contractile agonist instead of Carbachol.

pMLC expression is decreased with inhibiton of hMOF

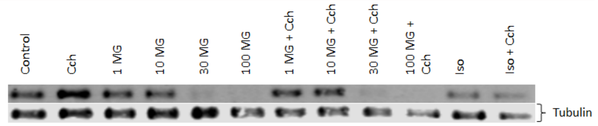


Figure 3. pMLC and Tubulin derived from N100217 donor HASM were assessed using Western blots, and a dose-dependent response to 1-100µM MG149 alone and 1-100µM MG149 with Carbachol (Cch) stimulation was observed with decreasing pMLC expression. This pattern is representative of the group mean. A similar dose-dependent response was observed with the use of Histamine as the contractile agonist instead of Carbachol.

Carbachol-induced pMLC is attenuated by EZH2 inhibition

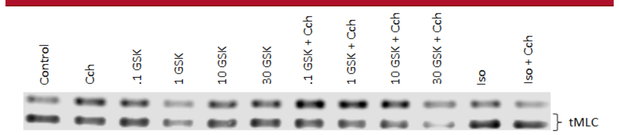


Figure 4. pMLC and tMLC derived from N050316 donor HASM were assessed using Western blots, and a small dose-dependent response to 10-100µM GSK126 with Carbachol (Cch) stimulation was observed with slight decreasing pMLC expression.

Agonist-induced pAkt is attenuated by hMOF inhibition

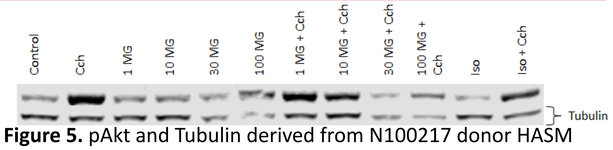


Figure 5. pAkt and Tubulin derived from N100217 donor HASM were assessed using Western blots, and a small increase was observed from 1-100µM MG149 alone. A dose-dependent response to 1-30µM MG149 with Carbachol (Cch) stimulation was observed with decreasing pAkt expression. This pattern is representative of the group mean. A similar pattern was observed with the use of Histamine as the contractile agonist.

Carbachol-induced pAkt is attenuated by EZH2 inhibition

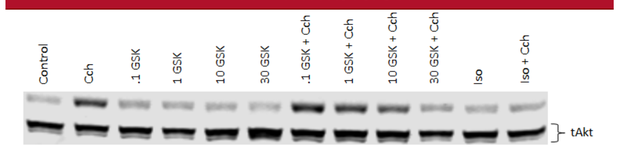


Figure 6. pAkt and tAkt derived from N050316 donor HASM were assessed using Western blots, and a dose-dependent response to 1-100µM GSK126 with Carbachol (Cch) stimulation was observed with slight decreasing pAkt expression.

pMYPT1 expression is decreased following inhibition of hMOF

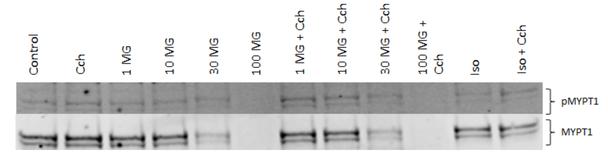


Figure 7. pMYPT1 and tMYPT1 derived from N112918 donor HASM were assessed using Western blots, and a dose-dependent response to 10-100µM MG149 alone and 10-100µM MG149 with Carbachol (Cch) stimulation was observed with decreasing pMYPT1 expression. This pattern is representative of the group mean. The same dose-dependent response was observed with the use of Histamine as the contractile agonist.

pMYPT1 expression is not significantly affected by EZH2 inhibition

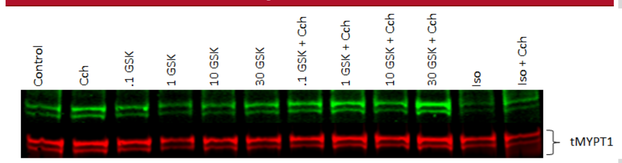


Figure 8. pMYPT1 and tMYPT1 derived from N100217 donor HASM were assessed using Western blots, and no pattern was observed for pMYPT1 expression to GSK126 treatment alone and GSK126 treatment with Carbachol (Cch) stimulation. However, a spike in pMYPT1 expression was observed at 30µM GSK126 alone and 30µM GSK126 with Carbachol.

Significance

The patterns indicated by the expression of EZH2, MLC, Akt, and MYPT1 proteins following MG149 treatment and contractile agonist stimulation suggest that inhibition of MOF, a histone acetyltransferase, can inhibit HASM hypercontractility. Therefore, a novel and interesting therapeutic target to attenuate asthma hyperresponsiveness may involve the modulation of histone acetylation. Protein expression following GSK126 treatment suggests that EZH2 is also a possible component of this pathway.

References

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Acknowledgements

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