Elevated Histone H3K27 Methylation Mediates Intrinsic Hypercontractility in Human Airway Smooth Muscle Cells From Subjects with Fatal Asthma

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Abstract

RATIONALE: Asthma manifests as airway hyperresponsiveness and inflammation. Importantly, human airway smooth muscle cells (HASMC) serve as the pivotal cell regulating bronchomotor tone. Whether an intrinsic abnormality in HASMC in asthma exists is controversial. Literature suggests that HASMC from subjects with asthma maintain a hypercontractile phenotype in culture. Since cell phenotype is epigenetically regulated, we hypothesize that histone modifications are altered in HASMC derived from fatal asthma subjects compared to non-asthma HASMC.

METHODS: HASM cells obtained from fatal asthma and non-asthma subjects were grown to confluencey. Cells were treated in the presence of a H3K27 methyltransferase inhibitor, UNC1999 (10 nM - 1 uM), or vehicle for 48 h. Lysates were subject to quantitative histone modification analysis utilizing mass spectrometry. Carbachol and histamine-induced myosin light chain (MLC) phosphorylation were measured by immunoblot. Agonist-induced intracellular calcium flux was determined using fluo-8 dye and fluorescence microscopy.

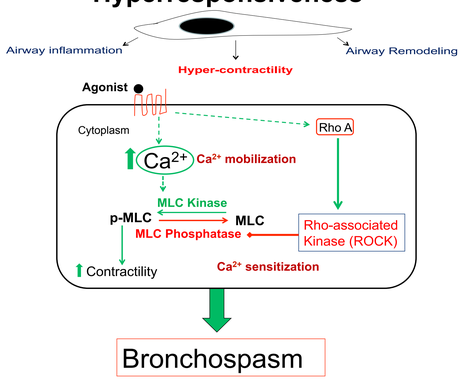
RESULTS: Histone H3K27 methylation was elevated in asthma HASMC as compared to non-asthma HASMC (62%, p=.016, n=5). H3K9 methylation, H3K4 methylation, and H4K16 acetylation were also elevated in asthma HASM. UNC1999 treatment suppressed global H3K27 methylation (3-fold compared to vehicle, p<.01, n=3) in non-asthma HASMC. Interestingly, UNC1999 treatment significantly blunted carbachol and histamine-induced calcium flux and myosin light chain phosphorylation (95%, p<.05, n=3). However, UNC1999 treatment had little effect carbachol and histamine-induced Rho kinase activation or total MLC expression.

CONCLUSIONS: H3K27 methylation is elevated in HASMC from subjects with asthma when compared to non-asthma, suggesting a distinct epigenetic signature between the two cell types. Inhibition of histone H3K27 methylation attenuated agonist-induced MLC phosphorylation and calcium mobilization, suggesting a role for epigenetic modulation of the contractile status of the cell. These findings suggest that H3K27 methylation is an important contributor to hyperresponsiveness observed in asthma and may serve as a new therapeutic target in treatment of allergic airway diseases

Epigenetic Regulation of Human Airway Smooth Muscle

- Human airway smooth muscle cells derived from fatal asthma subjects maintain a hypercontractile phenotype *in vitro.*
- Persistance of a hyperresponsive phenotype *in vitro* alludes to epigenetic alteration of cells in asthma.
- Post-translational modification of histones contributes to epigenetic regulation of cell phenotype.

Airway Smooth Muscle in Airway Hyperresponsiveness



Hypothesis

Histone post-translational modification contributes to intrinsic hypercontractility in HASM derived from fatal asthma subjects.

Histone H3K27 Methylation is Elevated in Asthma HASM

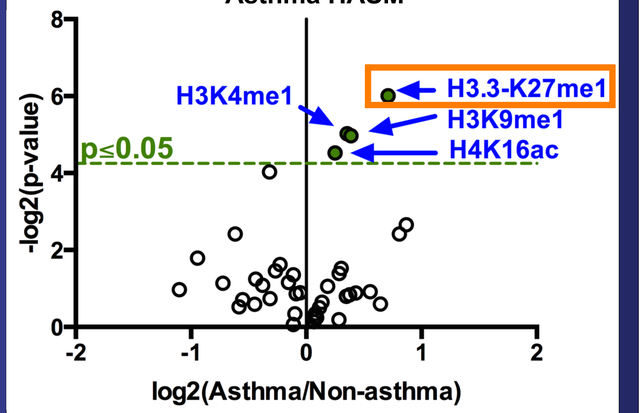


Figure 1– Human airway smooth muscle cells obtained from fatal asthma and non-asthma subjects were grown to confluence. Cells were lysed and subjected to mass-spectrometry based histone PTM analysis. Green circles represent histone PTM that were significantly different between asthma and non-asthma HASMC P≤0.05 (n=5).

UNC1999 Suppresses Histone H3K27me via Inhibition of EZH2 Methyltransferase

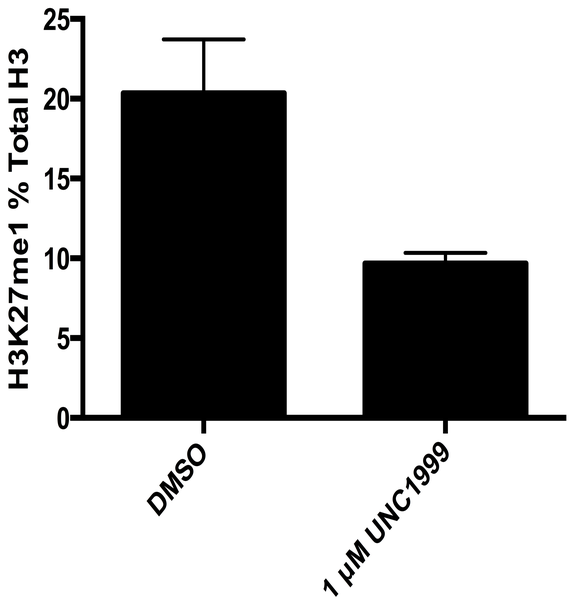


Figure 2– Human airway smooth muscle cells were grown to confluence and treated with UNC1999. Cells were lysed and subjected unbiased histone PTM analysis using liquid-chromatography/mass-spectrometry. Overall percentages of histone H3 with the lysine 27 either unmodified, monomethylated, dimethylated, or trimethylated (H3K27me1/2/3) following compound treatments (n=3).

UNC1999 Inhibits Carbachol-induced Myosin Light Chain Phosphorylation

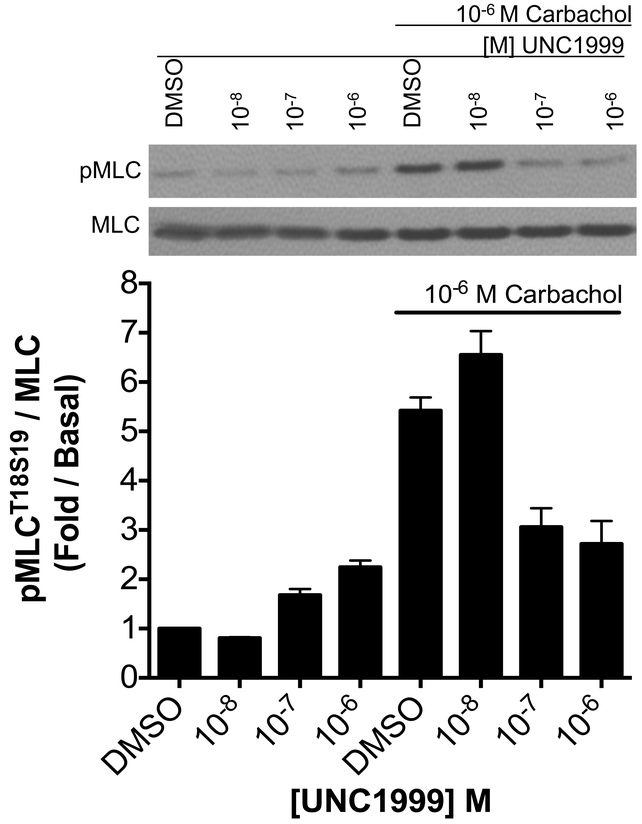


Figure 3– Human airway smooth muscle cells were treated for 48 h with UNC1999 and 10 m before lysis with carbachol. Cells were lysed and subjected to immunoblot. Membranes were probed with anti-pMLC, and MLC antibodies. Data shown as fold change of MLC phosphorylation as mean±SD (n=3).

UNC1999 Inhibits Agonist-induced Intraceullar Calcium Mobilization

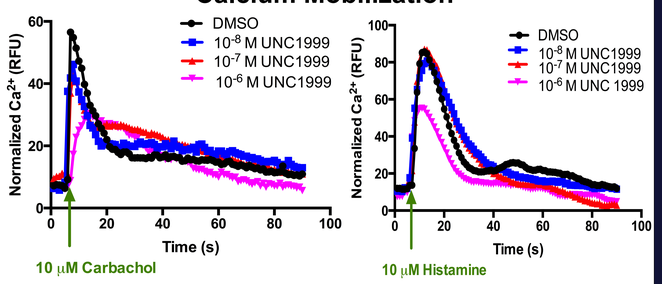


Figure 4– Human airway smooth muscle cells were treated for 48 h with UNC1999. Cells were loaded with Fluo-8 dye and stimulated with 10 μM carbachol. Fluoresence was measured using confocal microscopy. Data shown as relative fluoresence units mean±SD (n=9).

UNC1999 Inhibits Bronchoconstriction of Human Precision-Cut Lung Slices

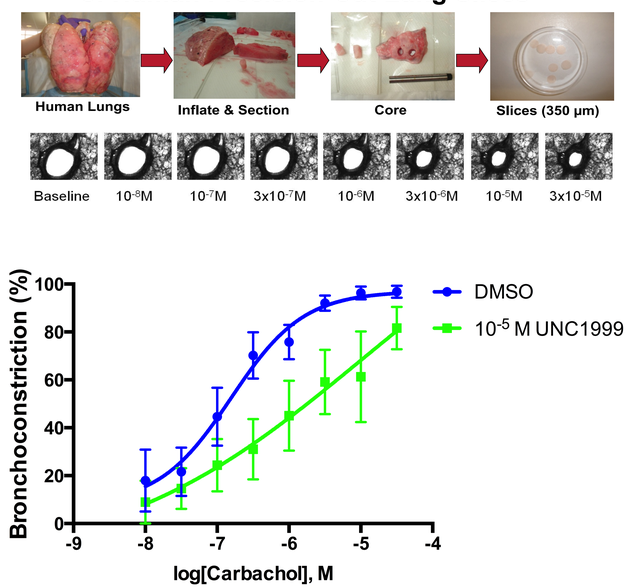


Figure 5– PCLS from normal healthy human donors, each containing a small airway, were treated with 10^{-5} M UNC1999 or DMSO. PCLS were bronchoconstricted to a dose response of carbachol (10^{-8} – 10^{-5} M). Data displayed as mean±SEM of 3-5 slices per donor (n=3).

Conclusions

1. Histone H3K27me is elevated in asthma-HASM.
2. UNC1999 suppresses histone H3K27 methylation.
3. UNC1999 inhibits myosin light chain phosphorylation.
4. UNC1999 inhibits agonist-induced intracellular calcium.
5. UNC1999 inhibits bronchoconstriction of hPCLS.

Significance

H3K27me can be an important contributor to hyperresponsiveness and may serve as a new therapeutic target in the treatment of allergic airway disease.

References

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