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

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Abstract

Histone modifications are airway hyperresponsiveness and inflammation. Importantly, human airway smooth muscle cells (HASM) serve as the primary cell model regulating bronchoconstriction. Whether or not an intrinsic awareness to inflammatory stimuli exists is controversial. Literature suggests that HASM from subjects with asthma exhibit a hyperresponsive phenotype in culture. Given cell phenotyping, we hypothesized that formaldehyde exposure of human small airways alters contractile responsiveness to contractile stimulus, without eliciting inflammatory mediator secretion.

CONCLUSIONS:

RESULTS:

METHODS:

RESULTS: Histone H3K27 methylation was elevated in asthma HASM as compared to non-asthma HASM (60%, p<0.005). H3K27M methylation and H3K9me1 were also elevated in asthma HASM. UNC1999 treatment suppressed global H3K27 methylation (40% compared to vehicle p<0.01) in normal HASM. Immune-UNC1999 treatment significantly altered calcium and histone-dependent calcium flux and human light chain phosphorylation (30%, p<0.05, M). However, UNC1999 treatment had little effect on calcium and histone-induced Rho kinase activation in total MLC expression.

CONCLUSIONS: H3K27 methylation is elevated in non-asthma HASM with subjects with asthma who compared to normal 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 regulation of the hypertrophic response. These data further support that H3K27 methylation is an important contributor to hypercontractility observed in asthma and may serve as a new therapeutic target in treatment of asthma airway disease.

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Hypothesis

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

Histone H3K27 Methylation is Elevated in Asthma HASM

UNC1999 Inhibits Carbachol-induced MLC Light Chain Phosphorylation

UNC1999 Inhibits Bronchoconstriction of Human Precision-Cut Lung Slices

Epigenetic Regulation of Human Airway Smooth Muscle

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

Airway Smooth Muscle in Airway Hyper responsiveness

H4K3me1 % Total H3

Figure 1: Human airway smooth muscle cells derived from fatal asthma and non-asthma subjects were grown to confluence and treated with UNC1999. Cells were loaded with Fluo-8 dye and stimulated with 10⁻⁶ M carbachol. Fluorescence was measured using confocal microscopy. Data shown as mean±SEM of 3-4 dishes per group (p<0.05).

Figure 2: Hypothesis post-translational modification contributes to intrinsic hypercontractility in HASM derived from fatal asthma subjects.

Figure 3: Human airway smooth muscle cells were grown to confluence and treated with UNC1999. Cells were loaded with Fluo-8 dye and stimulated with 10⁻⁶ M carbachol. Fluorescence was measured using confocal microscopy. Data shown as mean±SEM of 3-4 dishes per group (p<0.05).

Figure 4: Human airway smooth muscle cells were treated for 48 h with UNC1999. Cells were loaded with Fluo-8 dye and stimulated with 10⁻⁶ M carbachol. Fluorescence was measured using confocal microscopy. Data shown as mean±SEM of 3-4 dishes per group (p<0.05).

Figure 5: PCLS from normal healthy human donors, each containing a small airway, were treated with 10⁻⁶ M UNC1999 or DMSO. PCLS were bronchoconstricted to a dose response of carbachol (10⁻⁶ to 10⁻⁴ M). Data displayed as mean±S.E.M of 3-5 slices per donor (p<0.05).

Figure 6: Bronchial smooth muscle cells were treated for 48 h with UNC1999. Cells were loaded with Fluo-8 dye and stimulated with 10⁻⁶ M carbachol. Fluorescence was measured using confocal microscopy. Data shown as mean±SEM of 3-4 dishes per group (p<0.05).

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


