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Claudin 5 Transcripts Following Acrolein Exposure Affected by Epigenetic Enzyme

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Abstract

Background and objective: Acrolein is a highly reactive α, β-unsaturated aldehyde and a respiratory irritant that is ubiquitously present in the environment but that can also be generated endogenously at sites of inflammation. Apical junctional protein claudin 5 can be affected by acrolein. This study aimed to determine the impact of aberrant expression of DNMT1, DNMT3b, MBD3, and MeCP2 on acrolein induced claudin 5.

Methods: EA.hy926 cell lines were exposed to acrolein 30 nm for 1 h, 2 h, and 4 h. Epigenetic enzyme such as DNMT1, DNMT3b, MBD3 and MeCP2 were quantified in the cell line using real time PCR. Claudin 5 methylation was checked by methtyl specific PCR.

Results: After acrolein 30 nm exposure, MBD3 and MeCP2 transcript were decreased at 1 h and increased at 2 h and 4 h compared to control. DNMT3b transcript was decreased at 1 h, 2 h, and 4 h following acrolein 30 nm exposure. DNMT1 transcript was not different between control and acrolein 30 nm exposure. Claudin 5 methylation transcript /total Claudin 5 transcript was decreased at 1 h, 2 h and 4 h following acrolein 30 nm exposure.

Conclusion: These findings demonstrate that acrolein exposure modify epigenetic enzyme leading to Claudin 5 methylation change, suggesting that acrolein contribute to enzyme pathway involved in epigenetic regulation.

Keywords Acrolein; Epigenomics; Methylation; Claudin 5

Introduction

Acrolein (2-propenal) is a highly reactive α, β-unsaturated aldehyde and a respiratory irritant that is ubiquitously present in the environment but that can also be generated endogenously at sites of inflammation [1,2] . Acrolein is abundant in tobacco smoke, which is the major environmental risk factor for asthma and chronic obstructive pulmonary disease (COPD), and elevated levels of acrolein are found in the lung fluids of asthma and COPD patients [1-3]. Because of its reactivity with respiratory-lining fluid or cellular macromolecules, acrolein alters gene regulation, inflammation, mucociliary transport, and alveolar–capillary barrier integrity [1].

An integral membrane protein, Claudin 5 (CLDN5), is a critical component of endothelial tight junctions that control pericellular permeability [2]. Acrolein can induce ALI with perivascular edema in mice, accompanying by a compensatory increase in CLDN5 transcript and protein, which was more evident in a resistant than a sensitive mouse strain [2].

Epigenetics is the study of stable modifications of fixed genomes that direct which genes are expressed and silenced [4,5]. Although heritable from parent to child, and potentially stable between cell cycles, epigenetic regulation of DNA transcription can also be modified by a number of external factors to allow flexible responses to a changing environment such as air pollution, tobacco smoke, and other sources of oxidant stress, along with the microbial environment, pesticides, and toxins [4]. A number of different disease processes among them cancer, atherosclerosis, mental retardation syndromes, autoimmune, and allergic processes are in part controlled by epigenetic processes [4,6]. DNA methylation is accomplished by several subtypes of the DNA methyl transferases (DNMT). DNMT1 is considered the maintenance methyltransferase because this isoform acts to maintain methylation states during mitosis and in the daughter cells. DNMT3A and DNMT3B initiate de-novo methylation [7], although the triggers for this activity are only partly identified. Age, sex, genetic polymorphisms, and environmental exposures are some factors associated with altered methylation [8]. Histones can be modified by a number of processes including by acetylation, phosphorylation, methylation, ubiquitination or sumoylation [5]. This study aimed at examining the effect of epigenetic enzyme on claudin 5 expresion exposed to acrolein.

Materials and Methods

Cell culture and acrolein treatment

(No. CRL 2922; ATCC, Manassas, VA), Confluent cells were washed in D-PBS, incubated for 30 min, and then exposed to 30 nm acrolein (<4 h) in D-PBS.

Total RNA extraction and real-time PCR to measure methylation enzymes expression levels

Total RNA was extracted using a TRI REAGENT (Molecular Research Center, Cincinnati, OH, USA) according to the manufacturer's instructions. We quantified RNA and reverse transcribed cDNA from 3 μg of total RNA. RNA was reverse-transcribed by incubation with 0.5 mM dNTP, 2.5 mM $MgCl_2$, 5 mM DTT, 1 ul of Oligo DT(0.5 ug/ul) and SuperScript II RT (200 unit/ul) at 42°C for 50 min, and heat inactivated at 70°C for 15 min. About 50 ng cDNA was amplified using Applied Biosystems Step One TM Real-Time PCR System. The PCR mixture (20 μl) contained 1ul of cDNA, 1 μl of 10 pmol forward and reverse primers and 10 μl of 2X SYBR Green Supermix (Applied Biosystems). The reaction was carried out in a two-step procedure: denaturation at 95°C for 10 min and 40 cycles with denaturation at 95°C for 15 s, 60°C for 1 min and melt curve stage was performed at 95 for 15 s, 60 for 1 min and 90°C for 15 s. The comparative cycle number threshold (CT) method (ΔΔCT) was used to quantify the transcript expression levels. The change in CT value (ΔCT=CT gene of interest -CTPGK1) was calculated for each sample. The comparative ΔΔCT calculation involved finding the difference between each sample's ΔCT and the mean ΔCT for the control samples. These values were transformed to absolute values using the formula: comparative expression level (fold change)=2-ΔΔCT. The conditions and primers designed for detected genes were listed in Table 1.

Gene	Sequences (5'-3')		Annealing temperature (°C)	PCR products size (bp)
PGK1	F	GACCTAATGTCCAAAGCTGAGA	58	127
	R	A		
		CAGCAGGTATGCCAGAAGCC		
DNMT1	F	AACCTTCACCTAGCCCCAG	58	125
	R	CTCATCCGATTTGGCTCTTTCA		
DN MT3b	F	CCAATCCTGGAGGCTATCCG	60	152
	R	ACTGGGGTGTCAGAGCCAT		
MeCP2	F	TGACCGGGGACCCATGTAT	58	145
	R	CTCCACTTTAGAGCGAAAGGC		
MBD3	F	CAGCCGTGACCAAGATTACC	59	135
	R	CTCCTCAGCAATGTCGAAGG		

Table 1: Primer sets for real time PCR.

Claudin-5 CpG methylation in vitro by methylation-specific PCR (MSP) and real-time MSP

Total genomic DNA from the EA.hy 926 cells was extracted using a mini DNeasy Kit (Qiagen, Tokyo, Japan). Bisulfite conversion of genomic DNA was performed using a Zymo EZ DNA Methylation Gold kit (Zymo Research Corp, Orange, CA). MSP was performed to determine the methylation status of the CLDN5 gene. Specific methylated or unmethylated sequences of the primers: Human Claudin-5-M methylated primers forward: GTAAGGTGTTTTTGGAATGATTTC reverse: ATCCAACCACCAATCTTAATACG Human Claudin-5-U unmethylated primers forward: GTAAGGTGTTTTTGGAAATGATTTT revers: ATCCAACCACCAATCTTAATACAAC. These primers designed using the Methprimer tool.

Methyl amplification used hot start premix (bionner, Daejeon, Republic of Korea) and conditions were as follows: initial denaturation at 95°C for 10 min, denaturation at 94°C for 30 s, annealing at 58°C for 30s, and extension at 72°C for 30 s for 35 cycles, followed by a stabilization for 7 min at 72°C. Unmethyl amplification followed the same procedure. Amplification products were separated by gel electrophoresis and stained with ethidium bromide. The density (intensity 3 square millimeters) of each band specific for methyl and unmethyl primers was measured under UV light and promoter methylation status was expressed by percent density of methyl band / [methyl band+unmethyl band].

Statistical analysis

All data were analyzed using the SPSS version 7.5 for Windows. Data are expressed as mean ± SEM. Inter-group comparisons were assessed by non-parametric method using Mann-Whitney U test. A p-value of less than 5% was regarded as statistically significant.

Results

DNMT3b transcript decrease after exposure to acrolein

EA.hy926 cell lines were exposed to acrolein 30 nm for 1 h, 2 h, and 4 h. Epigenetic enzyme such as DNMT1, DNMT3b, MeCP2 and MBD3 were quantified in the cell line using real time PCR. DNMT3b transcript was decreased at 1 h, 2 h, and 4 h following acrolein 30 nm exposure (Figure 1A). DNMT1 transcript was not different between control and acrolein 30 nm exposure (Figure 1B).

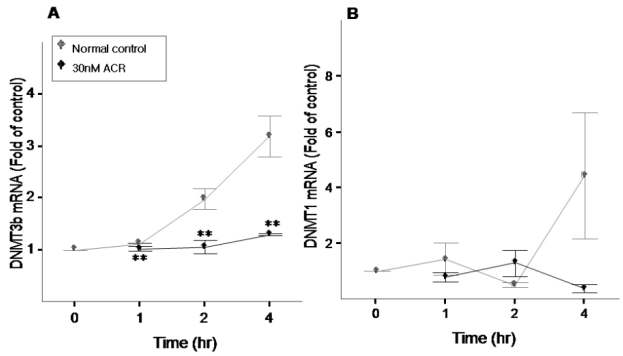

Figure 1: Changes of DNMT3b (A) and DNMT1 (B) following acrolein 30 nm exposure by time. Data showed mean ± SE. **p<0.05 compared to control.

Changes of MeCP2 and MBD3 transcript exposed to acrolein

After acrolein 30 nm exposure, MeCP2 (Figure 2A) and MBD3 (Figure 2B) transcript were decreased at 1 h and increased at 2 h and 4 h compared to control.

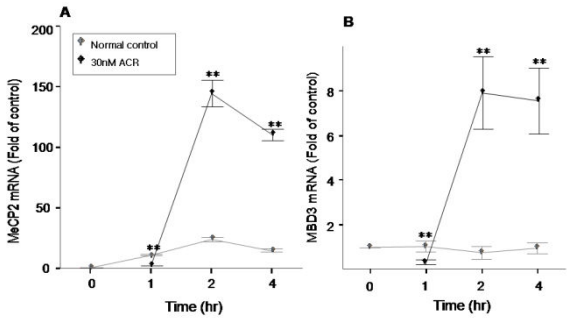

Figure 2: Changes of MeCP2 (A) and MBD3 (B) following acrolein 30 nm exposure by time. Data shows mean ± SE. **p<0.05 compared to control.

Claudin 5 methylation transcript decrease after exposure to acrolein

Claudin 5 methylation was checked by methtyl specific PCR. Claudin 5 methylation transcript/total Claudin 5 transcript was decreased at 1 h, 2 h, and 4 h following acrolein 30 nm exposure (Figure 3).

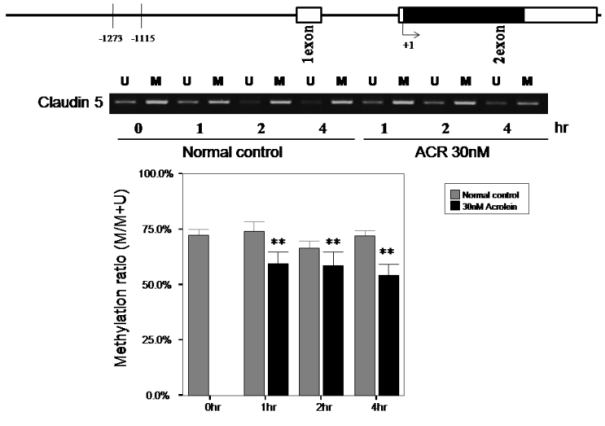

Figure 3: Changes of methylation/unmethlation and methylation ratio of claudin-5 following acrolein 30 nm exposure by time. Data showed mean ± SE. **p<0.05 compared to control.

Discussion

This study adds to novel information that acrolein exposure modify differently epigenetic enzyme by time windows. Acrolein (an α, β-unsaturated 2-alkenal) is highly reactive in biological systems and can be extremely irritating [9-11]. Acrolein levels are elevated in second-hand smoke compared with mainstream smoke because side-stream smoke is generated at lower combustion temperatures [12-14].

Epigenetics refer to inheritable changes beyond DNA sequence that control cell identity and morphology and play key roles in development and cell fate commitments and highly impact the etiology of many human diseases such as respiratory diseases [4]. DNA methylation, histone modification, and miRNAs represent coordinated processes that regulate gene silencing or expression by architectural remodeling of the genome [4].

Epigenetic changes are modulated by environmental exposures such as air pollutants, making epigenetics the interface between genes and environment [4]. With more knowledge about acrolein effect on human effects, it may be the window through which we can control exposures to protect patients from environmental exposure. In our study claudin 5 methylation ratio (methylation/unmethylation plus methylation) was decreased at 1 h, 2 h, and 4 h following acrolein 30 nm exposure indicating that methylation of claudin-5 gene decrease gene expression.

DNA methylation has been shown to be a key contributor to epigenetic regulation of gene expression. Its mechanism of action can be mediated through direct inhibition of transcription factors or DNA interactions modulated by methylation of specific regions of promoters [15-19], recognition of methylated DNA sequences by transcriptional repressors associated with the recruitment of corepressors [20,21], or binding of methylated DNA binding proteins (MBPs) to nucleosomes leading to chromatin compaction, as shown with methyl-CpG-binding protein 2 (MeCP2) [22].

Understanding how the methylome is affected by environmental signals that do not affect methyl donor levels is more challenging. Aging [23], smoking [24,25], and heavy metal exposure [26,27], acrolein [28,29] have all been associated with changes in the DNA methylome, and with changes in DNMT expression, but the mechanisms by which these changes occur are largely unknown. DNA methyltransferases (DNMTs) such as DNMT1, DNMT3A and DNMT3B contribute to various physiologic and pathologic conditions including embryo implantation [30], cytomegalovirus infection [31], radiation [32], cancer, aging, neural cell differentiation [33]. In this study although DNMT3b was increased at 1 h, 2 h, and 4 h, and DNMT1 was not changed compared to control in acrolein 30 nm exposure. Those results suggest that acrolein exposure affect DNMTs enzymes according to acrolein concentration.

MeCP2 (Methyl-CpG-binding protein 2) was the first MBP discovered to selectively recognize and bind methylated DNA sequences [34]. Since the discovery of MeCP2, 4 additional members of the MBP family (MBD1, MBD2, MBD3, and MBD4) have been identified through bioinformatic analysis of the polypeptide sequence of the methylated DNA binding domain (MBD) shared by these proteins [35].

MeCP2 was also the first MBP found to interact with HDAC-containing complexes, linking two epigenetic repression mechanisms: DNA methylation and histone deacetylation [36]. After acrolein 30 nm exposure, MBD3 and MeCP2 were decreased compared to control at 1 h and increased at 2 h and 4 h, indicating that MBD and MeCP2 differently function by time and dose following acrolein exposure.

In conclusion our study demonstrate that acrolein exposure modify MBD 3 and DNMTs and MeCP2 related to epigenetics, suggesting that acrolein contribute to enzyme pathway involved in epigenetic regulation. Further studies will be needed to explain the role of specific gene related to acrolein exposure.

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