

Expression of thioredoxin-binding protein-2/vitamine D3 upregulated protein-1 in peripheral blood eosinophils of asthma patients

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Abstract: Objective To investigate the expression of vitamine D3 upregulated protein-1 (VDUP1) in peripheral eosinophils at different stages of asthma and its correlation with clinical manifestations of asthma. **Methods** Fourteen normal volunteers and 51 mild to moderate asthma patients, including 16 untreated patients with asthma attack and 35 with asthma remission by continuously corticosterone inhalation. The symptom severity and pulmonary function indices were evaluated and induced sputum eosinophil counts and blood eosinophil count measured. VDUP1 and β -actin gene fragments were amplified simultaneously by RT-PCR from the total RNA of peripheral eosinophils, and 10 μ l of the RT-PCR product underwent agarose gel electrophoresis and the VDUP1/ β -actin ratio was obtained by Gel-Pro software. **Results** VDUP1/ β -actin ratio significantly decreased in untreated patients with asthma attacks in comparison with normal volunteers (0.314 ± 0.242 vs 0.532 ± 0.279) but not in patients with asthma remission (0.612 ± 0.381). In the former patients, a positive correlation of VDUP1/ β -actin ratio was found with FEV1.0% ($r=0.587$, $P=0.046$) and %PEF ($r=0.563$, $P=0.033$), whereas an inverse one observed with sputum eosinophil count ($r=-0.436$, $P=0.049$). **Conclusion** VDUP1 expression in the eosinophils correlates to eosinophil activation and may influence the disease severity of asthma patients.

Key words: thioredoxin-binding protein-2/vitamin D3 upregulated protein-1; thioredoxin; bronchial asthma; eosinophils, activation forced expiratory volume

Bronchial asthma is a chronic airway inflammation involving such immune cells as T lymphocytes and eosinophils^[1, 2], and eosinophil activation is much highlighted for its crucial role in this allergic inflammatory response. The eosinophils are recruited from circulation into the inflammatory site by chemoattractants and cytokines including platelet-activating factor, interleukin (IL)-5, IL-3, etc, which also prolong eosinophil survival both in the circulation and the inflammation foci, and prime eosinophils for degranulation and releasing toxic metabolites such as eosinophil granule proteins and reactive oxygen species (ROS), to cause epithelial damage. Once activated by cytokines or chemokines, the eosinophils may contribute to asthma by secreting cytokines and ROS^[3-5].

The molecular mechanism of modulation in asthma has currently remained unclear. To identify differentially expressed genes in the eosinophils in relation to asthma, we constructed previously two subtracted cDNA libraries for asthma attack-related

genes and asthma suppressor genes of human peripheral eosinophils by suppression subtractive hybridization (SSH) and screened totally 38 differentially expressed cDNA clones including 27 known genes, among which 25 were upregulated in the eosinophils during acute stage of asthma. Vitamin D3 upregulated protein (VDUP)-1/thioredoxin (TRX)-binding protein 2 is one of these known genes.

In this study, we investigated the expression of VDUP1 in peripheral blood eosinophils derived from healthy volunteers and asthma patients using RT-PCR, and examined the correlation between VDUP1 expression and the clinical features of asthma.

MATERIALS AND METHODS

Subjects

Blood samples were collected from 14 normal volunteers (Group A) and 51 patients with mild to moderate asthma including 16 with asthma attack but not having received any treatment (Group B) and 35 in remission by continuous corticosterone inhalation (Group C). All the 51 patients were diagnosed according to asthma diagnostic criteria of the American Thoracic Society^[6]. None of the patients with asthma had complications of other lung diseases or a history of allergy. The severity of asthma and asthma attack were

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classified according to the NHLBI/WHO Workshop in the Global Strategy for Asthma (GINA guideline)^[7]. Written informed consent to participate in the study was obtained from all the volunteers and patients who were provided with detailed information concerning the study and their rights, and this study was approved by the ethical committee of Southern Medical University.

Purification of peripheral blood eosinophils and total RNA extraction

The lymphocytes were removed from the venous blood of asthma patients and healthy volunteers using lymphocyte isolation reagent kit (Dingguo Biotechnology Co.Ltd., Beijing), and the red blood cells by cell lysis with ammonium chloride. The eosinophils were isolated from the granulocytes on Percoll (Amersham Pharmacia Biotech) discontinuous density gradient centrifugation, with the purity of approximately 95% as determined with Diff-Quick staining. The total RNA was extracted using Trizol Reagent (Invitrogen Life Technologies, American), chloroform and isoamyl alcohol (Guanghua Chemical Factory Co. Ltd., China) following the manufacturer's instructions.

RT-PCR protocol

The first-strand complementary DNA (cDNA) was generated by reverse transcription (RT) of the mRNA at 42 °C for 60 min, and used for amplification of VDUP1 gene and β -actin gene (serving as internal control) through polymerase chain reaction (PCR) with TaKaRa RNA PCR Kit (AMV) Ver 3.0 (TaKaRa Biotechnology Co. Ltd., Japan). The sense primer 5'GCAATCATATTATCTCAGGGAC3' and antisense primer 5'CATCTCAGAGCTGGTTCG3' were used to generate the 236-bp VDUP1 PCR products. The sense primer 5'GAAATCGTGCGTGACAATAA3' and antisense primer 5'GTACTTGCGCTCAGGAGG3' were used to generate the 393-bp β -actin PCR products. The PCR amplification was performed in 35 cycles of denaturation at 94 °C for 45 s, annealing at 50 °C for 1 min, and elongation at 72 °C for 2 min. After the amplification, 10 μ l of the PCR products were run on 1% agarose gel. The bands of amplified β -actin were used to normalize the cDNA from the samples. The images of agarose gel electrophoresis were analyzed by Gel-Pro software to obtain the VDUP1/ β -actin ratio.

Evaluation of pulmonary function

Immediately after the blood sampling, the

percentage of forced expiratory volume in one second (FEV1.0) in its predicted value (FEV1.0%) and percentage of predicted peak expiratory flow (%PEF) were determined using Master Screen IOS (Hoechberg, Germany).

Induced sputum eosinophil count

All the subjects were asked to inhale 3% hyperosmotic saline through efflux nebulizer for 5-15 min and saliva was removed from 10 ml induced sputum on petri dish, in which 10 ml 10% DTT was added and mixed with the sputum through repeated pipette. Leucocytes cytometry and cell classification were performed with HE staining.

Statistical analysis

Normally distributed data were expressed as $Mean \pm SD$. Differences of VDUP1/ β -actin among the groups were tested by one-way ANOVA. Correlations of VDUP1 expression in the eosinophils with pulmonary function (FEV1.0% and %PEF) and sputum eosinophil count were evaluated with Pearson's correlation coefficient. A *P* value less than 0.05 was considered to indicate significant statistical difference.

RESULTS

Expression of VDUP1 in ensinophils

To study the role of VDUP1 in asthma, we compared its expression in peripheral blood ensinophils (Fig.1) from both healthy controls and asthma patients in different stages. The results revealed a significant decrease of the ratios of VDUP1/ β -actin in the 16 untreated patients with asthma attacks (0.314 ± 0.242 , $P=0.049$) in comparison with the normal volunteers (0.532 ± 0.279 , $P<0.05$). In contrast, no significant difference was noted between patients in remission (0.612 ± 0.381 , $P=0.076$) and healthy volunteers. (Fig.2).

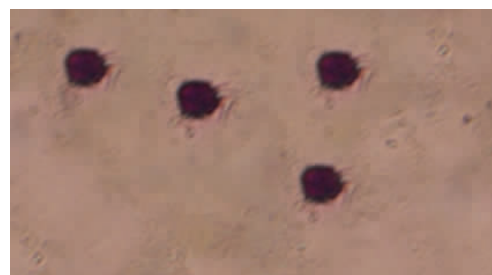


Fig.1 Peripheral blood eosinophils of a patient with asthma attack (Wright's staining, 10 \times 40)

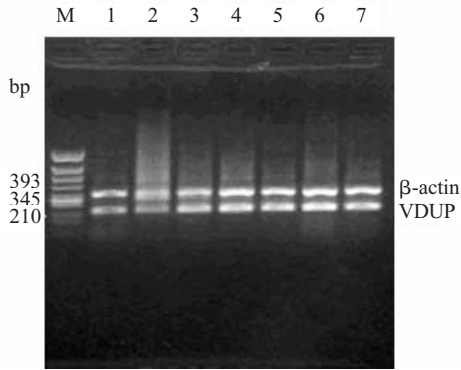


Fig.2 Agarose gel electrophoresis of the RT-PCR product of VDUP1 in the eosinophils from asthma patients and healthy volunteers

M: DNA marker; Lanes 1-3: Asthma patients in remission by glucocorticosteroid inhalation; Lane 4: Healthy volunteer; Lanes 5-7: Asthma patients with attack

Relation between eosinophil VDUP1 expression and pulmonary function

In untreated patients with asthma attacks, a positive correlation of the ratios of VDUP1/ β -actin was established with FEV1.0% ($r=0.587$, $P=0.046$) and %PEF ($r=0.563$, $P=0.033$), whereas the ratio of VDUP1/ β -actin was not correlated to either FEV1.0% or %PEF in healthy volunteers or asthma patients in remission (Tab.1).

Tab.1 Relation between VDUP1/ β -actin ratio and pulmonary function

	Group A (n=14)		Group B (n=16)		Group C (n=35)	
	FEV1.0%	PEF%	FEV1.0%	PEF%	FEV1.0%	PEF%
<i>r</i>	0.271	0.280	0.587	0.563	0.331	0.348
<i>P</i>	0.380	0.310	0.046	0.033	0.160	0.120

Relation between VDUP1 expression and sputum eosinophil count

The sputum eosinophil counts were 0.004 ± 0.003 in normal volunteers, 0.612 ± 0.110 in patients with asthma attack and 0.275 ± 0.079 in asthma patients in remission treated with glucocorticosteroid inhalation. No correlation was observed between VDUP1/ β -actin ratio and sputum eosinophil count in healthy volunteers ($r=0.147$, $P=0.053$) or in asthma patients in remission ($r=-0.412$, $P=0.051$). In untreated patients with asthma attack, however, an inverse correlation was observed between VDUP1/ β -actin ratio and sputum eosinophil count ($r=-0.436$, $P=0.049$).

DISCUSSION

We investigated VDUP1 expression in the eosinophils and its correlation with clinical manifestations of asthma patients in different phases. Lower VDUP1 expression was found in untreated patients with acute asthma episodes as compared with that in healthy volunteers, whereas patients with controlled asthma by steroids treatment showed basically normal VDUP1 expression. In addition, we found that VDUP1 expression was positively correlated with the severity of airway obstruction but inversely correlated with induced sputum eosinophil count in patients with asthma attacks.

The maintenance of cellular redox balance relies heavily on the activity regulation of several antioxidant systems, among which TRX system (TRX, TRX reductase, and NADPH) is ubiquitous and currently known to possess both intracellular and extracellular activities and reduce ROS through an interaction with the redox-active center of TRX. TRX exhibits antioxidant activity that regulates redox-sensitive molecules such as nuclear factor (NF)- κ B, activator protein 1 (AP-1), and glucocorticoid receptors inside the cells^[8, 9], and influences gene expression by oxidoreductive modification of the transcription factors through the modulation of cellular redox status^[10]. TRX also has extracellular cytokine- or chemokine-like activities and regulates cell proliferation and apoptosis^[9, 11, 12]. Serum TRX level in patients with asthma attack correlates with the pulmonary function and eosinophil cationic protein (ECP)^[13]. ROS induces eosinophil apoptosis and simultaneously activates the antioxidant systems, including TRX system for maintaining survival and normal function of the cells^[14].

VDUP-1/TRX-binding protein is an endogenous inhibitor regulating TRX function^[15]. Overexpression of VDUP1 suppresses TRX protein expression and its reducing activity, and therefore VDUP1 participates in the pathophysiological process of several diseases by modulating cellular redox state. ROS induces down-regulation of VDUP1 in vascular smooth muscle cells to diminish the negative regulation of TRX, resulting consequently in hyperplasia of the smooth muscles. Inversely, excessive oxidative stress or shearing stress leads to passive expression of VDUP1 in the myocardial cells to inhibit TRX, which undermines the survival of the myocardial cells^[16, 17]. VDUP1 expression is also stimulated

by hyperglycemia in myocardial cells or smooth muscle cells and lead to concurrent disease in cardiovascular system originated from hyperglycemia^[18]. It is thus obvious that VDUP1 expression induced by ROS, hyperglycemia and fluid shear stress in the myocardial cells and vascular smooth muscular cells impacts on the expression and function of TRX to the end of oxidoreductive regulation, with therefore intimate involvement in the pathologic progress of hypertension, atherosclerosis, cardiac hypertrophy and so on.

In previous studies we identified VDUP1 as one of the differentially expressed genes in peripheral blood eosinophils from a patient who experienced acute asthma episodes and remission. In this study, we further verified the differential expression of VDUP1 in eosinophils in asthma patients, suggesting the involvement of VDUP1 in the activation and functional regulation of the eosinophils in asthma. VDUP1 expression in eosinophils of untreated patients with asthma attacks was found positively related to FEV1.0% and % PEF, which are important indices for airway obstruction, but inversely related to sputum eosinophil count which indicates asthmatic airway inflammation in direct association with disease severity of asthma^[19, 20]. As VDUP1 functions as the endogenous inhibitor of TRX and is involved in the modulation mechanism of the pathological processes of hypertension, atherosclerosis and cardiac hypertrophy, we speculate that VDUP1 might affect the expression and activation of TRX, which activates the eosinophils and prolongs their survival.

The exact mechanisms of the effect of VDUP1 in eosinophils are unclear. The important protective role of TRX in the cells has inspired attempts of clinical therapy for many diseases with TRX as a promising target. Obviously, the investigation of VDUP1, the endogenous suppressor of TRX, can be also significant for devising new therapeutic strategies. VDUP1 has been known as one of the metastasis suppressor in breast cancer^[21] and 5-FU may induce its expression in tumor cells^[22], whose proliferation and survival is related to VDUP1 down-regulation. A novel strategy of cancer therapy lies in up-regulating VDUP1 expression so as to inhibit the function of TRX^[21,22], and similarly, controlling the expression of VDUP1 in the eosinophils may also serve as a possible solution for asthma therapy.

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硫氧还蛋白结合蛋白 -2/ 维生素 D3 上调蛋白 1 在哮喘病人外周血嗜酸性粒细胞的表达

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摘要:目的 探讨硫氧还蛋白结合蛋白 / 维生素 D3 上调蛋白 1 (VDUP1) 在不同时期哮喘病人嗜酸细胞中的表达及其与哮喘临床症状的关系。方法 抽取正常自愿受试者 14 例, 急性发病未经任何治疗的 16 例轻到中度哮喘病人和通过吸入糖皮质激素而处于哮喘缓解期的 35 例病人的外周静脉血 15 ml 进行外周血嗜酸细胞计数和诱导痰嗜酸细胞计数, 随后行肺功能检查测定 FEV1.0% 和 PEF%。分离纯化静脉血嗜酸细胞, 提取总 RNA。RT-PCR 同时扩增特异性片段 VDUP1 和内参 β -Actin。取 10 μ l 反应物进行 1% 琼脂糖凝胶电泳, 用 Gel-Pro 软件分析凝胶成像, 测定 VDUP1 和 β -Actin 灰度值, 求 VDUP1/ β -Actin 比值, 并比较不同组别之间 VDUP1 表达的变化, 分析 VDUP1/ β -Actin 比值与诱导痰嗜酸细胞、FEV1.0%、PEF% 的相关性。结果 急性期未经治疗的哮喘病人嗜酸细胞 (0.314 \pm 0.242) 与正常人比较 (0.532 \pm 0.279) 显著降低, 而经吸入糖皮质激素处于缓解期哮喘病人嗜酸细胞 VDUP1/ β -Actin 比值 (0.612 \pm 0.381) 与正常对照组无显著差异。在急性发作未经治疗哮喘组嗜酸细胞 VDUP1 表达与 FEV1.0% ($r=0.587$, $P=0.046$) 和 PEF% ($r=0.563$, $P=0.033$) 呈正相关, 而与诱导痰嗜酸细胞计数呈负相关关系 ($r=-0.436$, $P=0.049$)。结论 嗜酸细胞 VDUP1 的表达与哮喘嗜酸细胞活化相关, 并影响哮喘病人的临床症状。

关键词: 维生素 D3 上调蛋白 1 硫氧还蛋白结合蛋白 哮喘 嗜酸性粒细胞 FEV1.0% PEF%

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