HDL Inhibited Atherosclerosis Induced by Radiation Injury

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Research

Keywords: Atherosclerosis, radiation, endothelial cells, high-density lipoprotein

Posted Date: December 3rd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-118806/v1

Version of Record: A version of this preprint was published at ScienceAsia on January 1st, 2022. See the published version at https://doi.org/10.2306/scienceasia1513-1874.2022.013.

Abstract

Background- HDL inhibits atherosclerosis development from radiation damage; however, the mechanism for this process has not been fully defined.

Methods- This study used radiation patients and cultured mouse aortic endothelial cells (MAECs) to investigate the process. Firstly, 158 patients from oncology department of Jingzhou hospital who have got radiation after neck cancers, the arterial function was monitored by B ultrasound, HDL and other blood lipid indexes were tested.Then, MAECs were isolated and cultured, MTT assay was used to test the HDL protective role on UVB radiation, along with this, western blotting was proceed to test some apotosis protein expression and possible molecular.

Results- Firstly, those patients with high HDL levels were less likely to develop atherosclerosis, with statistical differences. We observed that MAECs treated with UVB was damaged significantly; HDL reversed the cell damage as dose-depended manner. At mean time, the apoptosis process was assessed and found that HDL inhibited the apoptosis caused by UVB. Western blotting results showed that HDL enhanced phosphatidylinositol 3-kinase (PI3K) and Akt phosphorylation in MAECs.

Conclusion- These results suggest that HDL protected UVB-mediated appotosis by activation of a mechanism involving PI3K/Akt signal pathway.

Introduction

Atherosclerosis is a disorder occurring in the large arteries and the primary cause of heart diseases [1]. It can make the arterial wall hard, the lumen narrow, the elasticity of the middle membrane weakened, and lead to serious complications including ischemic heart disease (IHD), myocardial infarction (MI), stroke (including cerebral thrombosis and cerebral hemorrhage), gangrene of limbs, etc.Many factors are considered to be related to the occurrence and development of atherosclerosis. Hyperlipidemia is the main risk factor of atherosclerosis [2]. Compared with the patients of the same age and same sex who have no hypertension, the incidence of atherosclerosis is earlier. Smoking is one of the risk factors of atherosclerosis, and it is also the main independent risk factor of coronary heart disease. Some research results show that a large number of smokings can lead to vascular endothelial cell damage, along with increasing of carboxyhemoglobin [3]. Diabetes and hyperinsulinemia are important complications of atherosclerosis. Diabetes and hyperinsulinemia are related to secondary hyperlipidemia. The higher the level of insulin in blood, and the lower the content of HDL, the higher the morbidity and mortality of coronary heart disease[4]. Family history genetic factors atherosclerosis is a strong independent risk factor. The incidence of atherosclerosis in patients with familial hypercholesterolemia and familial lipoprotein lipase deficiency was significantly higher than that in the control group[5, 6]. According to the age-related pathological data, atherosclerosis is a slow developing process from infancy. The detection rate and severity of atherosclerosis increase with age, which is related to the age-related changes of arterial wall. Before menopause, the incidence of coronary atherosclerosis in women was lower than that

in men of the same age group, the level of HDL was higher than that in men, and the level of LDL was lower than that in men. After menopause, the difference between the sexes disappeared, which may be related to the effect of estrogen[7]. Life or medical radiation exposure can cause damage to the body, including atherosclerosis.Radiation in high altitude area causes atherosclerosis [8]; Radiation exposure increases the risk of coronary artery disease (CAD) including atherosclerosis, also this was related with the radiation dose [9]. This is to assess the risk of atherosclerosis caused by coronary angiography and the decisions taken by patients [10]. Radiation treated esophageal cancer patients also increase the risk of heart complications encompass mainly pericardial disease, cardiomyopathy, coronary artery atherosclerosis, valvular heart disease, and arrhythmia[11].So, how to avoid the risk in imaging examination or radiotherapy was in urgent.

HDL is recognized to prevent or alleviate atherosclerosis through many pathways [12, 13].whether the HDL can also promote the atherosclerosis caused by radiation and the mechanism is not clear. In this study, firstly we check the relationship between the ratio of atherosclerosis in radiation patients and the plasma lipid level; then we use MAECs treated by radiation to study the HDL role and possible mechanisms.

Material And Methods

2.1. Patients

158 patients from oncology department of Jingzhou hospital who have got radiation after neck cancers, the arterial function was monitored by B ultrasound, HDL and other blood lipid indexes were tested. All patients provided written informed consent.

2.2. Materials

C57BL/6 J mice were used for MAECs isolation. DMEM medium was from Gibco Company. All animal experiments were approved by the Institutional Animal Care and Use Committee.The Ethics Committee of Soochow University approved he present study protocol.

2.3. Cell culture

MAECs were isolated from C57BL/6 J mice using an outgrowth technique as described previously [14, 15]. MAECs were maintained in DMEM medium with 10% calf serum at 37 °C in a humidified incubator supplemented with 5% carbon dioxide. For all experiments, MAECs cells were grown to near-confluence, then serum-starved overnight (12–16 h) in serum-free DMEM medium.

2.4. Prepare of HDL

Following the procedure, HDL was prepared [16]. Briefly, Human serum obtained from Jingzhou central hospital (Jingzhou, Hubei) was overlaid with a potassium bromide (KBr) gradient solution at a density of 1.063 g/ml. Low- and very low-density lipoproteins were removed from these samples by ultracentrifugation at 35,000 rpm for 18 h. The infranatant was adjusted to 1.21 g/ml with solid KBr and

mixed with 1.21 g/ml buffered KBr solution, and then centrifuged at 48,000 rpm for 48 h. HDL was collected and dialysed against three changes of phosphate buffer saline (PBS) with 1 mM EDTA at 4°C for 48 h. Final dialysis was against PBS without EDTA for 8 h, followed by filtration through a 0.22-mm filter.

2.5. MTT assay

The cell growth was measured by MTT assay. Cells were seeded at a density of 5×10^3 cells/well in a 96-well plate and cultured for indicated time intervals. At each time interval, the medium was replaced with fresh cell culture medium containing 0.5 mg/mL MTT for 4 h. Add 150 ul DMSO to each well and shake on the low speed rotation for 10 min until the crystal is fully dissolved, then measured at 490 nm by a Multiskan MS ELSA reader (xMark Microplate Absorbance Spectrophotometer, BioRad, USA). The relative cell number was normalized by the absorbance from the control cells.

2.6. SDS-PAGE and Western blotting

The total protein was used for Western blot assay following the standard Western blotting protocol (Molecular Clone, Edition II).The concentrations of the primary antibodies employed were: PI3K (1:1500, Santa Cruz,sc-374534), p-ERK(1:500, Santa Cruz,sc-7383), ERK(1:500, Santa Cruz,sc-94), p-AKT(1:500, Santa Cruz,sc-7985R), AKT(1:500, Santa Cruz,sc-8312), cleaved caspase 3(1:500, CST,9579), ATF-4(1:500, abcam,ab-25331)and GAPDH (1:1000, Santa Cruz, sc-575). Respective horseradish peroxidase (HRP) labeled secondary antibodies were applied and enhanced chemiluminescence (ECL) detection was used according to the manufacturer's instructions (Pierce, Rockford,IL). The integrated density mean grey value of the band was analyzed under ImageJ software and the corresponding relative expression ratio was calculated.

2.7. Statistical analysis

Dates are expressed as means ± SE. The differences of means between multiple groups was assessed with the two tailed Student's t-test (for 2 groups) and the analysis of variance (ANOVA, for > 2 groups). $P \leq 0.05$ was considered statistically significant for all analyses.

Results

3.1. HDL level related with the ratio of atherosclerosis

118 patients (74.7%) of all radiation patients were found to have atherosclerosis with varying degrees. Atherosclerosis incidence rate was not significantly associated with sex, but with age. Plasma lipid test results showed that Non-AS patients had higher HDL level than AS patients, indicated that high HDL levels were less likely to develop atherosclerosis, with statistical differences. (Table 1)

Table 1
Relationship between incidence of atherosclerosis and blood lipid

Parameters	AS	Non-AS	X2	*p value*
Sex (Female/male)	39/79	15/25	0.102	0.749
Age (≤ 50/>50)	30/88	26/14	18.756	< 0.001
Total cholesterol	7.38 ± 0.82	4.25 ± 0.74	21.364	< 0.001
Triglyceride	3.85 ± 0.68	2.46 ± 0.65	11.295	< 0.001
LDL	3.57 ± 0.54	2.83 ± 0.48	7.695	< 0.001
HDL	1.35 ± 0.43	1.97 ± 0.57	7.226	< 0.001

3.2. HDL reversed radiation-cell damage

As radiation exposure with 90μw/cm^2 ultraviolet rays for 10 minutes, the MAECs then were treated with 50, 100, 200 μg/ml HDL or not, MTT results showed that UVB caused about 52% MAECs damage, but HDL reversed the phenomenon, this indicated that HDL protected MAECs damage caused by UVB, Fig. 1.

3.3. HDL protected UVB-induced apoptosis

Compared with control group or HDL group, cleaved caspase 3 expression in the UVB group was increased; But the UVB group then treated by 200 μg/ml HDL, the cleaved caspase 3 expression was decreased, this indicated that HDL alleviated UVB-induced apoptosis. ATF-4 as apoptosis protein was related with apoptosis induced by endoplasmic reticulum stress, in this study ATF-4 expression was increased by UVB and then reversed by HDL; this told us that HDL may be protect MAECs by inhibiting endoplasmic reticulum stress apoptosis, Fig. 2.

3.4. HDL inhibited radiation-induced atherosclerosis by PI3K/AKT signal pathway

To further explore the possible mechanism, this experiment was designed to study the impact of HDL on PI3K and Akt expression and phosphorylation. As shown in Fig. 3, UVB decreased the levels of total PI3K and Akt proteins; HDL treatment did not alter the levels of total PI3K and Akt proteins, but increased the phosphorylation level of these two proteins. However, inhibition of PI3K and Akt by LY294002. These results suggest that activation of PI3K-Akt pathway is a mechanism by which HDL induces cholesterol transport in MAECs, Fig. 3.

Discussion

Atherosclerosis cause many clinic problems such as angina, myocardial infarction, stroke, hemiplegia, aphasia, renal artery stenosis or lower extremity artery stenosis. These hazards to human body are quite

serious, which can lead to death. There are many reasons for atherosclerosis; also radiation may be a potential factor because some head and neck tumor patients get radiation or some radiologic patient has got atherosclerosis [17, 18]. HDL was considered as a very good protects to anti atherosclerosis, but whether the HDL also can protect radiation-induced atherosclerosis not fully consider. Our study showed that after radiation, the cell damaged seriously, but after the HDL pretreated, the cell damage can be reversed. Although some studies suggested that moderate ultraviolet radiation can prevent cardiovascular disease [19]. Low dose of ionizing radiation will increase the risk of cardiovascular disease, mainly cause endothelial cell damage and participate in the process of atherosclerosis [20, 21].HDL plays an anti atherosclerotic role by reducing the deposition of cholesterol in tissues, thus limiting the occurrence and development of atherosclerosis [22].Our clinic studies showed that radiation exposure was high risk factor during atherosclerosis prevalence, HDL superior to reduce this risk. Radiation can induce apoptosis through oxidative stress, during the cell experiment, MAECs apoptosis increased and HDL may protect [23]. But our study showed that HDL inhibited endothelial cell apoptosis [24–26]. Although some studies have found that UVB radiation resulted endothelial cells appotosis mainly through inhibiting PI3K/AKT signal pathway[27, 28], HDL can protected endothelial cells through PI3K/AKT signal pathway[29], but nearly no study about whether HDL can protect endothelial cell caused by UVB radiation.In this study, HDL increased PI3K/AKT expression in UVB-treated group, but cannot eliminate PI3K/AKT inhibitor LY294002 effect, this was consist with the result that PI3K/Akt pathway is not only driver in HDL-mediated cell protection[30].Therefore, with further experimental confirmation, there is a high chance to translate current result for clinical use to prevent radiotherapy-induced atherosclerosis.

Abbreviations

HDL: high density lipoprotein; MAECs: mouse aortic endothelial cells; AS: atherosclerosis; PI3K: phosphatidylinositol 3-kinase; MI: myocardial infarction; CAD: coronary artery disease; ATF4: activating transcription factor 4

Declarations

Ethics approval and consent to participate

Our study was approved by the Ethics Committee of Soochow University. Animal experiments were approved by the Institutional Animal Care and Use Committee of the Soochow University. All animal experiments were performed in accordance with the institutional guidelines for the care and use of laboratory animals and that animals were anesthetized and killed using acceptable techniques.

Consent for publication

Not applicable.

Availability of data and materials

The data will be available on request.

Conflict of interest

none declared.

Funding

This study was supported by nature science foundation of Hubei province (**2017CFB786**), medical school Youth Fund of Yangtze University (YXYQ201411) and Jingzhou Science and Technology Bureau Project (**2017-93**).

Authors' contribution

HJ designed and supervised the study. XJ, and ZK processed the study. XJ, ZK, and HJ wrote the manuscript. WQY, ZP and PLH contributed to tables and figures.HJ revised the manuscript. HJ acquired funding.

Acknowledgements

Not applicable.

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Figures

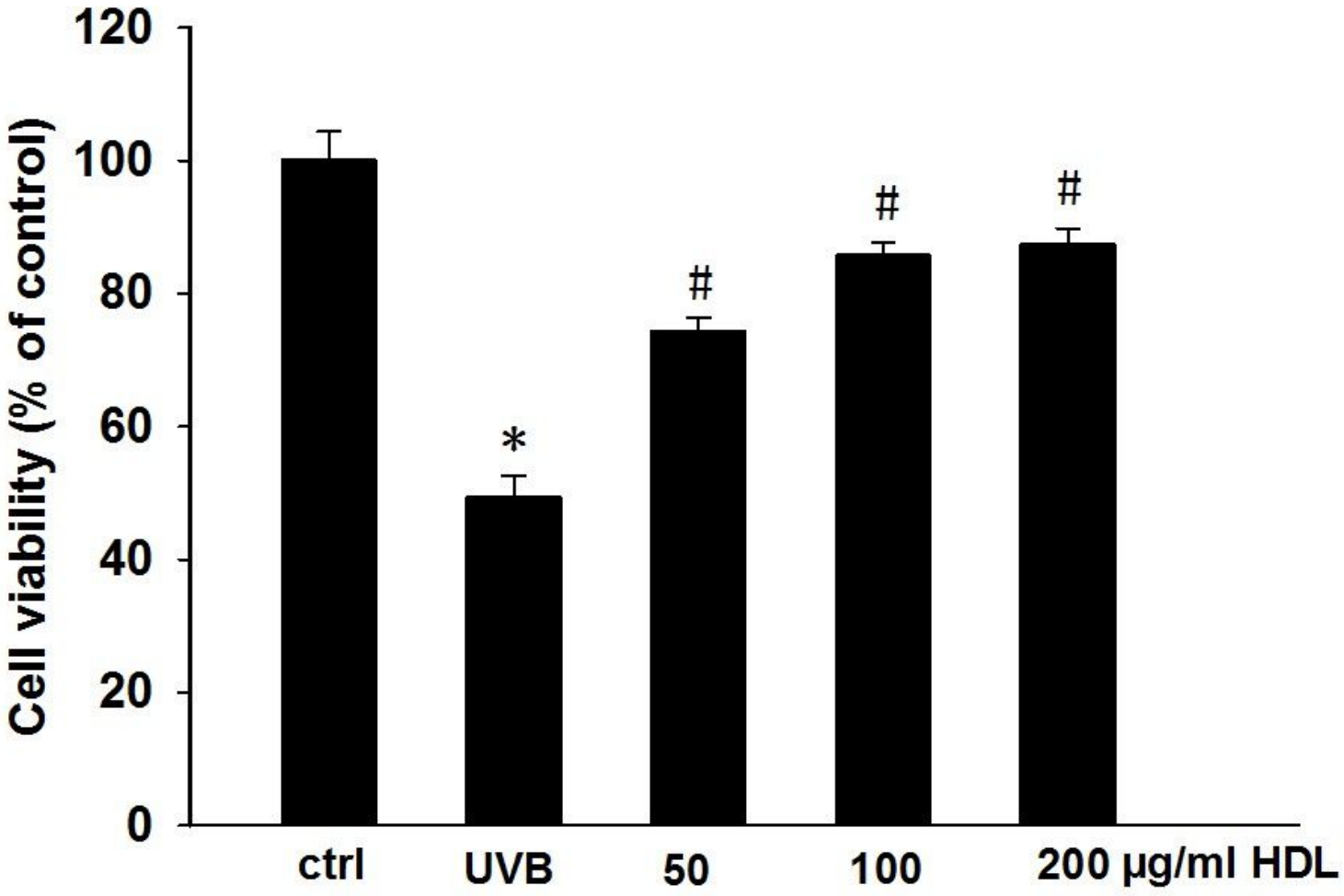
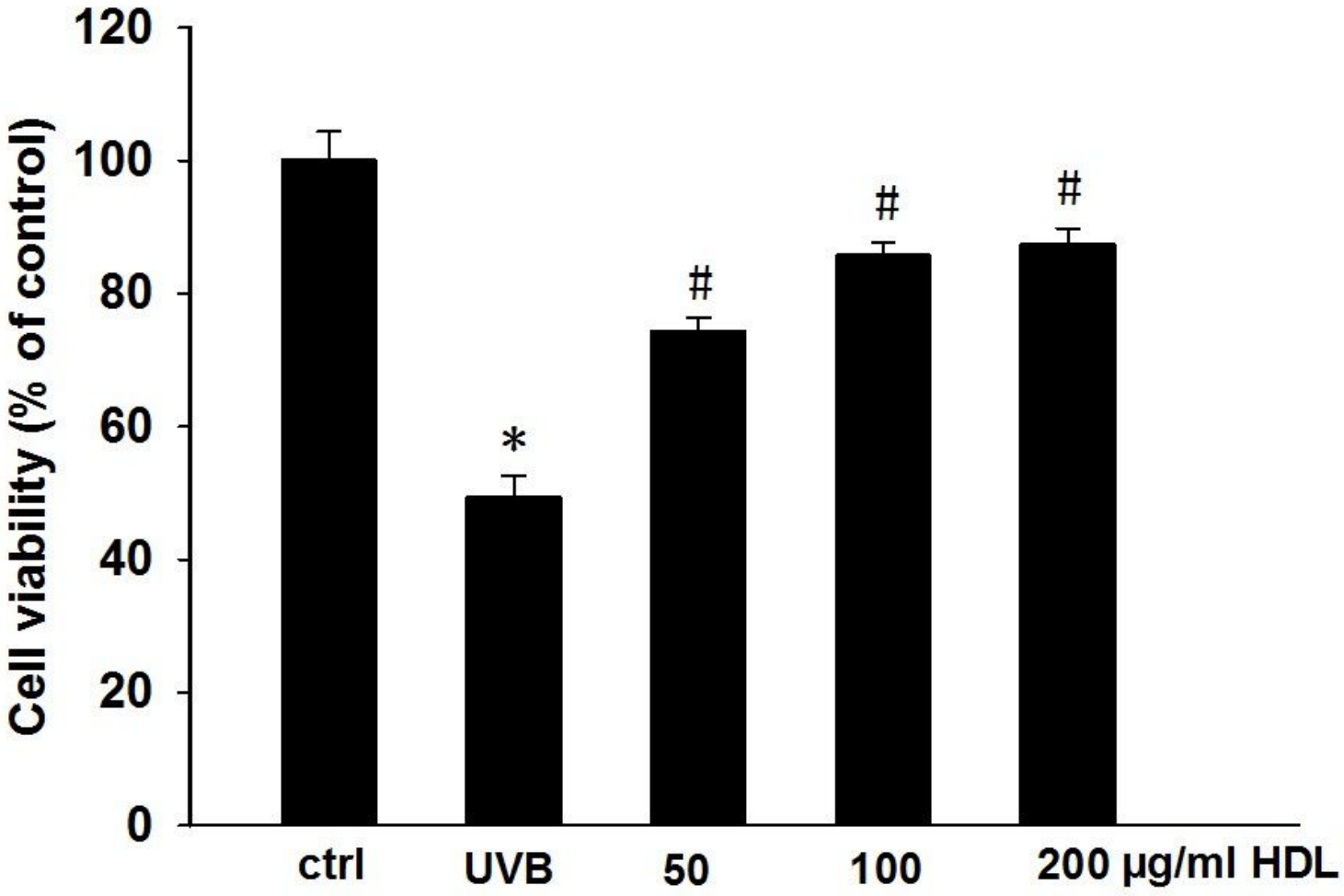


Figure 1

Effects of HDL on MAECs cell viability. Results were confirmed in three independent experiments. *compared with control group, p< 0.05; #compared with UVB group, p< 0.05.



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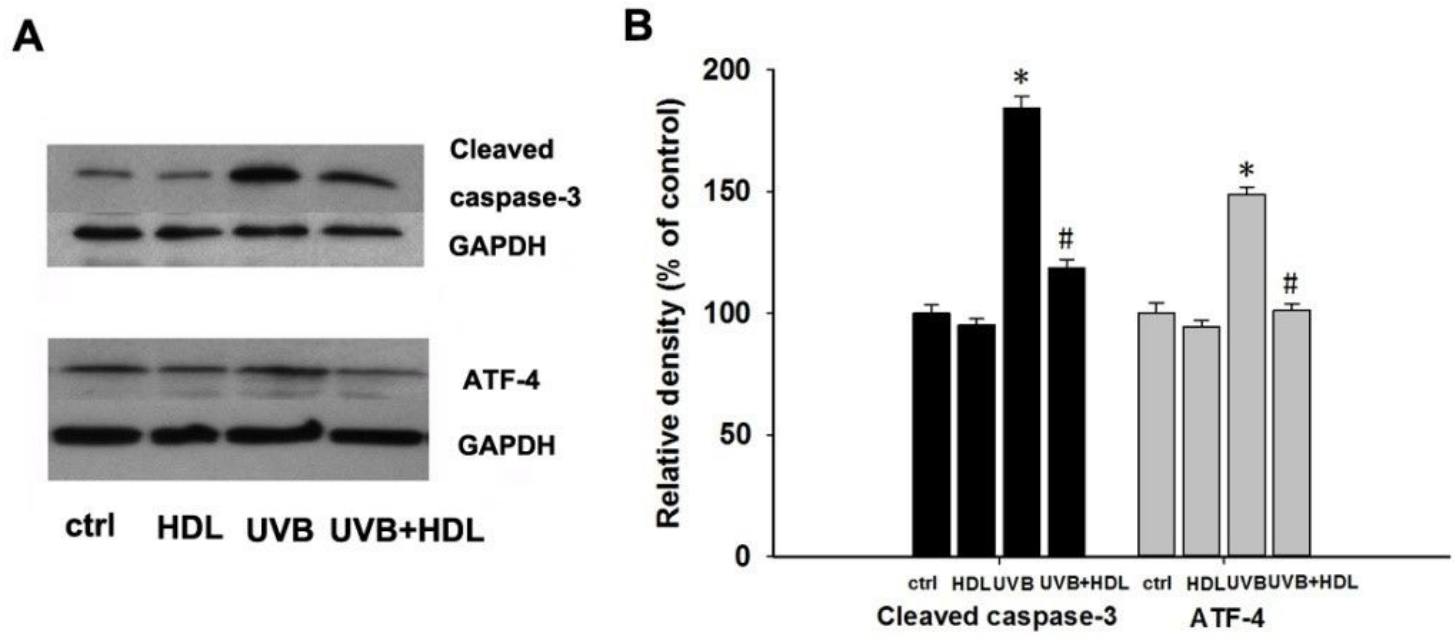
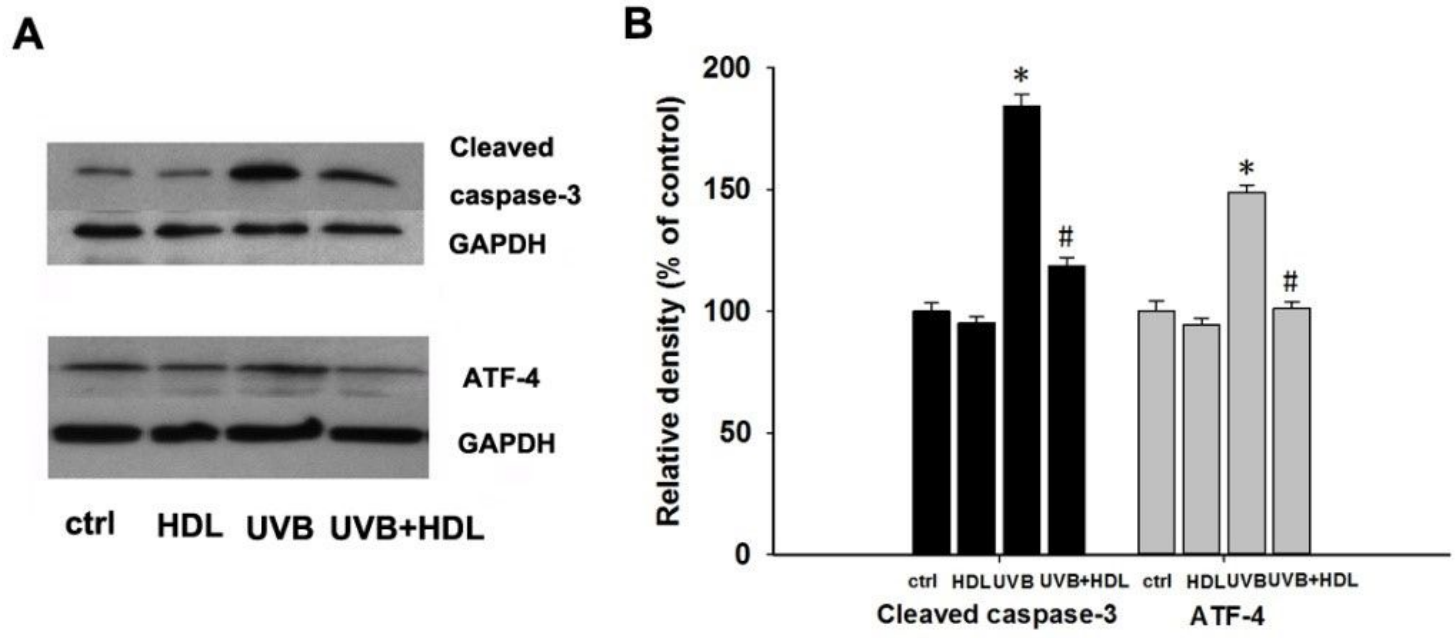


Figure 2

HDL inhibited MAECs apoptosis caused by UVB. *compared with control group, p< 0.05; #compared with UVB group, p< 0.05.



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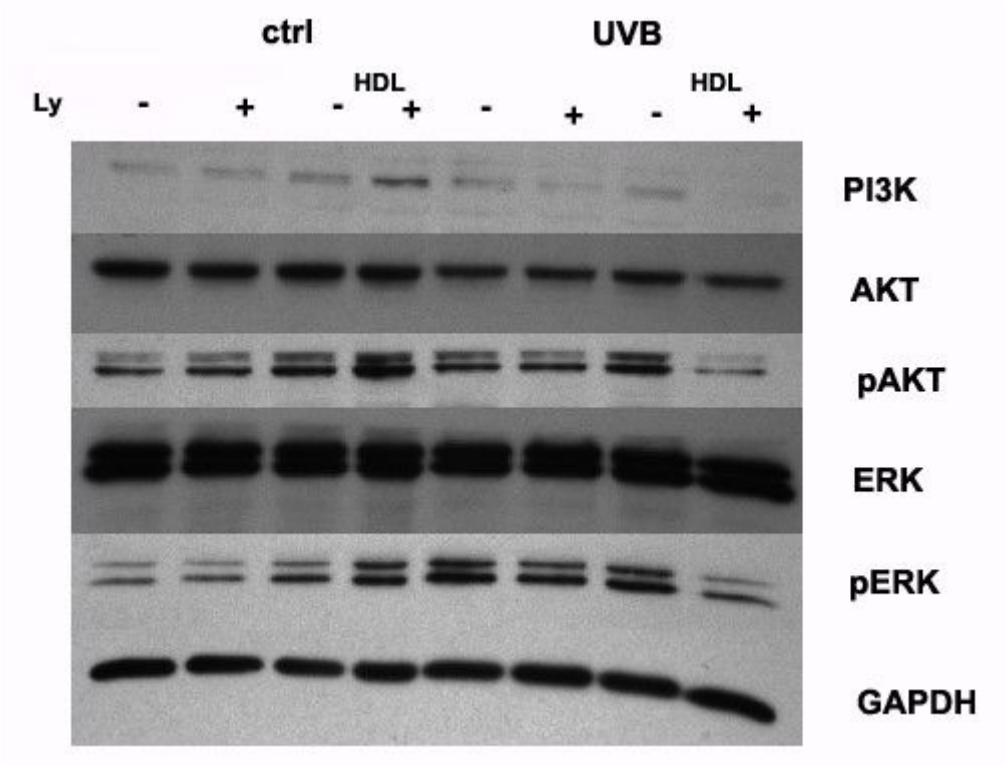
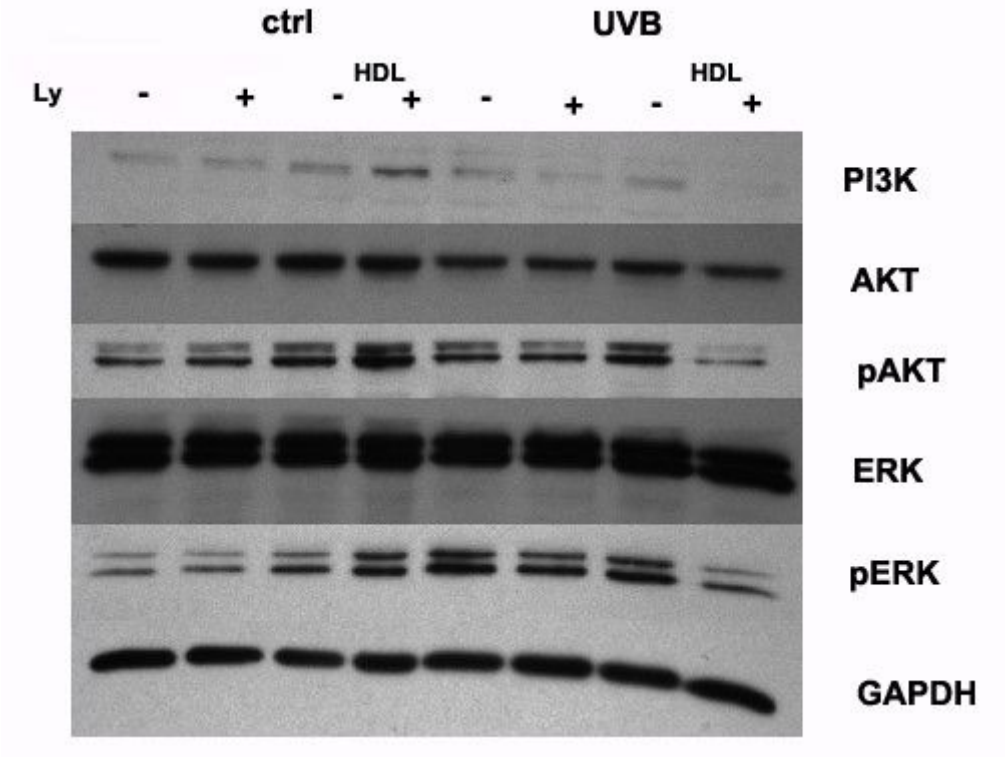


Figure 3

HDL inhibited MAECs apoptosis mainly through activation of PI3K/Akt pathway.



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