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Supplementary data

Title page

Role of VPO1, a newly identified heme-containing peroxidase, in ox-LDL induced endothelial cell apoptosis

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1. SUPPLEMENTARY MATERIALS AND METHODS

1.1 Materials

Fluorescent $^{-}$OCl (hypochlorite) detection kit was purchased from Cell Technology, Inc.(USA).

1.2 Detection of intracellular HOCl by APF and HPF

To further directly determine the intracellular generation of HOCl, we used fluorescent $^{-}$OCl (hypochlorite) detection kit. The two probes, aminophenyl fluorescein (APF) and hydroxyphenyl fluorescein (HPF), are cell permeable and can be used in combination to detect hypochlorite (-OCl) production in cells. Hypochlorite can be detected by loading two samples, one with APF and the other with HPF. Hypochlorite production is visualized by increase in fluorescence of APF loaded cells and no increase in fluorescence in HPF loaded cells. As described previously[1], endothelial cells were cultured in black, clear bottomed 6 well plates (Costar). On the day of the experiment, cells were washed twice in PBS and incubated in modified Hanks' balanced salt solution containing 10.0 mM HEPES, 1.0 mM $MgCl_2$, 2.0 mM $CaCl_2$, and 2.7 mM glucose adjusted to pH 7.3±0.05. The cells were loaded with HPF or APF (10 µM) for 30 min at room temperature. Dye-loaded cells were pre-treated with 600 µM apocynin or 10 µM DPI for 1 h, and then exposed to ox-LDL (100 µg/ml) for 30 min. Typical fluorescence images using Nikon Eclipse fluorescence microscope and quantification of fluorescence with fluorescence plate reader were obtained. The excitation and emission filters were 488 nm and 515 nm respectively.

SUPPLEMENTARY FIGURE LEGENDS

Figure S1 Detection of intracellular HOCl by APF and HPF probe. A and B, Intracellular HOCl level was determined by APF and HPF. C Typical fluorescence images which loaded with HPF or APF. Control, wild-type cells were incubated in modified Hanks' balanced salt solution for 30 min. ox-LDL (100 μg /ml), wild-type cells were treated with 100 μg/ml ox-LDL for 30min. +DPI, cells were pretreated with 10 μM DPI (the specific NADPH oxidase inhibitor) for 1h prior to ox-LDL exposure; + apocynin, cells were pretreated with 600 μM apocynin for 1h prior to ox-LDL exposure. +gp91^{phox} siRNA: after successful gp91^{phox} siRNA transfection, cells were incubated in modified Hanks' balanced salt solution containing ox-LDL (100 μg /ml) for 30 min; + VPO1 shRNA, after successful VPO1 shRNA transfection, cells were incubated in modified Hanks' balanced salt solution containing ox-LDL (100 μg /ml) for 30 min. gp91^{phox} siRNA: cells with successful gp91^{phox} siRNA transfection were incubated in modified Hanks' balanced salt solution for 30 min; VPO1 shRNA, cells with successful VPO shRNA transfection were incubated in modified Hanks' balanced salt solution for 30 min. DPI: cells were cultured in modified Hanks' balanced salt solution containing 10 μM DPI for 30 min; apocynin, cells were cultured in modified Hanks' balanced salt solution containing 600 μM apocynin for 30 min. $^{**}P < 0.01$ vs control (0 μg/ml ox-LDL), $^{++}P < 0.01$ *vs* ox-LDL (100 μg/ml). Data are expressed as mean ± SEM, n=6 each, performed in triplicate.

SUPPLEMENTAL REFERENCE

1. Setsukinai K, Urano Y, Kakinuma K, Majima HJ, Nagano T. Development of novel fluorescence probes that can reliably detect reactive oxygen species and distinguish specific species. *J Biol Chem.* **278**: 3170-5; 2003.

Figure S1