

Letter to the Editor

Expression of perilipin isoforms in cell types involved in atherogenesis

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Dear Editor,

In the January 2006 online issue of *Atherosclerosis*, Larigauderie et al. [1] reported about expression of perilipin in human macrophages (M Φ). The authors demonstrated an increased storage of triglycerides and formation of lipid droplets in M Φ overexpressing perilipin. Our group studied the expression of lipid droplet-associated PAT proteins (perilipin, adipophilin and TIP47) with freeze fracture immunocytochemical and electron microscopical techniques, but we were not able to detect perilipin in THP-1-derived M Φ or in human monocyte-derived M Φ (HMDM) [2,3]. Likewise Forcheron et al. [4] could not detect perilipin mRNA in THP-1 cells and HMDM using RT-PCR, except for a very low level found in some HMDM that had been incubated with acetylated LDL. Because of these contradictory results we examined the expression of perilipin in M Φ and other cell types involved in atherogenesis using western blot and immunohistochemistry.

Our western blot analysis of THP-1-derived M Φ , HMDM, human aortic smooth muscle cells (HASMC) and human umbilical vein endothelial cells (HUVEC) was performed with the same anti-perilipin A/B antibody as used by Larigauderie et al. [1]. In accordance with Larigauderie et al. [1], this analysis showed expression of perilipin protein in THP-1-derived M Φ and HMDM (Fig. 1A). Additionally, we found strong perilipin expression in HASMC, but no expression in HUVEC (Fig. 1A). Larigauderie et al. [1] detected only perilipin isoform A in THP-1-derived M Φ . In contrast to these findings, our western blots with THP-1-derived M Φ and HASMC protein revealed one single band at 46 kDa, indicating expression of perilipin B in these cell types. HMDM showed two bands at 58 and 46 kDa, indicating expression of both perilipin A and B (Fig. 1A). The different perilipin

isoforms A and B result from alternative splicing of a common pre-messenger RNA and knowledge about expression of the perilipin isoforms is of great interest, since they exert different functions. For example, perilipin A protects lipid droplets against protein kinase A-mediated lipolysis, whereas perilipin B does not [5]. Our *in vitro* experiments demonstrated expression of different perilipin isoforms in cell types involved in atherogenesis, but expression of perilipin isoforms in atherosclerotic lesions remained unclear. Therefore, we examined perilipin expression in the lysate of three different homogenized human coronary arteries with advanced atherosclerotic lesions. Western blots showed a strong perilipin A band (58 kDa) and a weak perilipin B band (46 kDa) (Fig. 1B), indicating that perilipin A is the predominant isoform of perilipin expressed in human atherosclerotic lesions. For future experiments investigating perilipin in atherogenesis we therefore encourage the use of perilipin A/B expressing HMDM rather than THP-1-derived M Φ which only express perilipin B. In addition to the above experiments, we examined five different coronary arteries with advanced lesions by performing immunohistochemistry. These specimens showed strong perilipin staining in regions of intimal M Φ and weak perilipin staining in regions of medial SMC, but no perilipin staining in regions of intimal SMC and endothelial cells (EC) (Fig. 1C). The results are in accordance with findings of Forcheron et al., who detected perilipin expression in CD68 and α -actin positive cells in human atheroma [4]. But in contrast to Faber et al. [6] we were not able to detect expression of perilipin in EC. These data suggest that perilipin might influence the metabolism of lipid droplets in M Φ -derived foam cells of advanced atherosclerotic lesions.

In additional experiments, we examined human coronary arteries with early and intermediate atherosclerotic lesions. Interestingly, we could detect perilipin expression neither in M Φ nor in SMC, whereas adipophilin, another member of the PAT protein family, colocalized with lipid-filled M Φ (data

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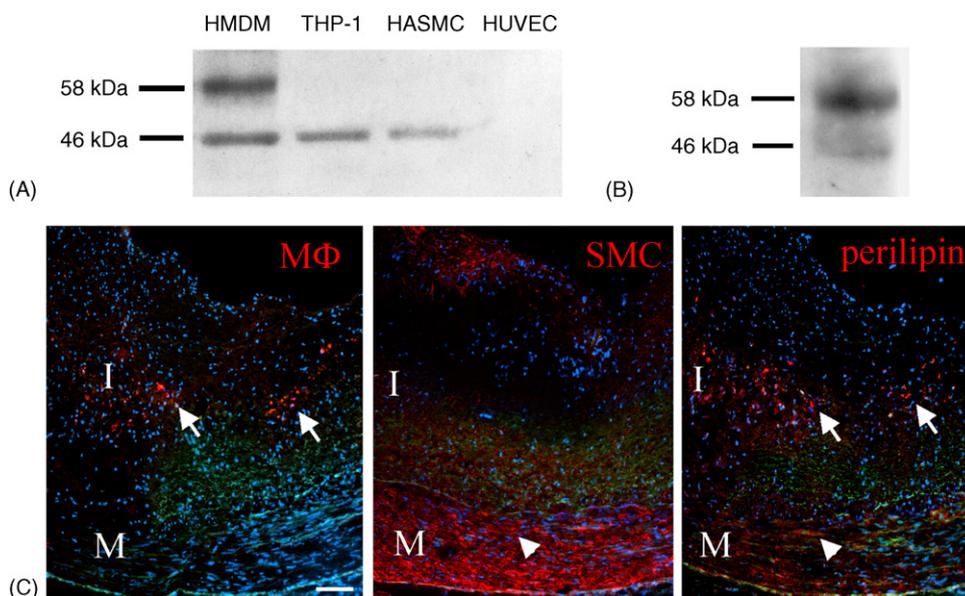


Fig. 1. Expression of perilipin in cultured cells and human coronary arteries. (A) Protein expression of perilipin in HMDM, THP-1-derived M Φ , HAOSMC and HUVEC. Western blots were performed using polyclonal guinea pig anti-perilipin A/B antibody (Progen) and peroxidase-conjugated anti-guinea pig antibody (Vector Laboratories) ($n = 3$). (B) Protein expression of perilipin in human coronary arteries. Coronary arteries were homogenized using the ball mill Mikro-Dismembrator S (Braun Biotech). Western blots were performed with the same antibodies as used in (A) ($n = 3$). (C) Immunohistochemistry of human coronary arteries. Serial cryosections were fixed with formaldehyde, blocked with BSA and incubated with monoclonal mouse anti-CD68 (M Φ) antibody (DAKO), monoclonal mouse anti-smooth muscle actin antibody HHF35 (DAKO) or polyclonal guinea pig anti-perilipin A/B antibody (Progen) followed by incubation with Cy3-conjugated secondary antibody (Chemicon). Nuclei staining was performed with Hoechst dye 33258. Red perilin staining can be found in regions with M Φ (arrows) and in the media (arrowhead). Blue: nuclei, green: autofluorescence, red: perilipin. M: media, I: intima. Bar: 100 μ m.

not shown). During differentiation of adipocytes, adipophilin, which is initially expressed, is replaced by perilipin. Similar to Larigauderie et al. [1], we suppose that this might also be valid for M Φ -derived foam cells in advanced lesions. Thus, adipophilin may be replaced by perilipin A during atherogenesis, inhibiting lipolysis in advanced lesions by stabilizing lipid droplets in these cells.

In summary, we encourage the use of perilipin A/B expressing HMDM rather than THP-1-derived M Φ for future experiments investigating perilipin in M Φ . Additionally we demonstrated that perilipin A may influence the metabolism of lipid droplets in M Φ -derived foam cells of advanced atherosclerotic lesions, perhaps by replacing the PAT family protein adipophilin. With regard to the protective effect of perilipin A against lipolysis, we suppose that perilipin A stabilizes the lipid droplets of foam cells in advanced atherosclerotic lesions. Explaining the presence of perilipin A in M Φ -derived foam cells and unravelling its functions is going to be a challenging task for the future.

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