SUPPLEMENTARY INFORMATION

FOR

MECHANISTIC INSIGHTS INTO THE ANTI-RESTENOTIC EFFECTS OF HSP27 AND HO1 MODULATED BY RECONSTITUTED HDL ON NEOINTIMAL HYPERPLASIA

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- Supplementary Methods
- Supplementary Tables 1 and 2
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SUPPLYMENTARY METHODS

Preparation of reconstituted HDL

In first step, 150 kg of precipitate IV of human plasma was washed with 3 volumes (w/v) of buffer (70 mM sodium citrate, 80 mM sodium phosphate, pH 4.0) for 2 hours and the precipitate was suspended in 4 volumes of 6 M urea for 2 hours at room temperature. The suspension was filtered and obtained a precipitate of 17-37% range by adding polyethylene glycol. The precipitate was suspended in 300 L of buffer (30 mM Tris-HCl, pH 8.0) and 600 L of ethanol. The suspension was filtered, the pH of the filtrate lowered to 6.0 and the resulting apoA-I precipitate was collected by further filtration. The precipitate was solubilized in 100 L of 4 M guanidine HCl and pasteurized for 10 hours at 60℃. The pH was then adjusted to 7.5 and applied to anion-exchange chromatography. The purified apoA-I was filtrated with 20 nm filter.

Subsequently, rHDL with a molar ratio of apoA-I to PC of 1:150 was prepared. The above apoA-I solution was mixed with lipid solution of 1.24 kg soybean PC and 715 g sodium cholate in 10 mM Tris-HCl, 10 mM NaCl, 1 mM EDTA, pH 7.5 for 12 hours at 5℃. After the mixture was diafiltered with at least 5 volumes of a 1% sucrose solution, sucrose was added to a final concentration of 10% and the concentration of the lipoprotein solution was adjusted to 20 mg/ml. After a final sterile filtration, the rHDL was filled in bottles of 1 g rHDL (protein weight) and lyophilized. The dry product was stored at -4℃ in the dark until used. Before infusion, the lyophilized product was reconstituted with non-pyrogenic water for injection. The rHDL showed a disc-shaped, noncovalently associated particles resembling nascent HDL (Fig.1).

SUPPLEMENTARY TABLES

Supplementary Table 1.

Primer name	Sequence (5'-3')
siHSP27-sense	GCUGCAAAAUCCGAUGAGAC=tt
siHSP27-antisense	GUCUCAUCGGAUUUUGCAGC=tt
siHO1-sense	CCUUGUACCAUAUCUAUAC=tt-
siHO1-antisense	GUAUAGAUAUGGUACAAGG=tt
Scrambled siRNA-sense	AUGAAACUGUUGUCAGCGCUG=tt
Scrambled siRNA-antisense	CAGCGCUGACAACAGUUUCAU=tt

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siRNA name	Sequence (5'-3')
GAPDH-Fwd	GAGAAGGCTGGGGCTCATTT
GAPDH-Rev	AGTGATGGCATGGACTGTGG
HSP27-Fwd	ACGGTCAAGACCAAGGATG
HSP27-Rev	TTATTACTTGGCGGCAGTCT
HO1-Fwd	GTGCTCAAAAAGATTGCCCA
HO1-Rev	ATCTTGCACTTTGTTGCTGG
VCAM1-Fwd	TACCCATTTGACAGGCTGGA
VCAM1-Rev	TTGCATTTCCAGAAAGGTGC
CCR2-Fwd	CCACATCTCGGTTTATCAG
CCR2-Rev	CGTGGAAAATAAGGGCCACAG
CCR5-Fwd	GCAACATGCTGGTCATCCTC
CCR5-Rev	ACAGCCCTGTGCCTCTTCTT
CCR7-Fwd	CACAGTGCTCTCCATCCCAG
CCR7-Rev	CCGATGAAGGCGTACAAGAA
CX3CR1-Fwd	TCCGCAATGTGGAAACAAAT
CX3CR1-Fwd	ACTTCCATGCCTGCTCCTTT

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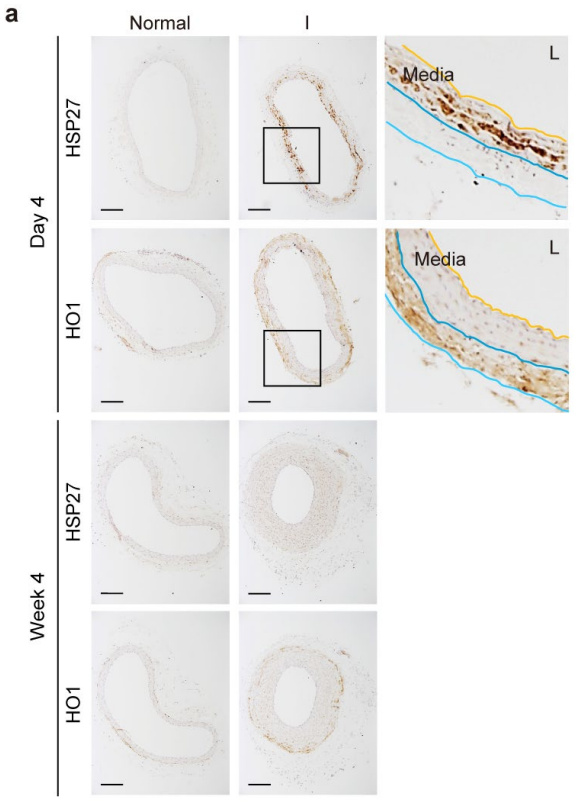
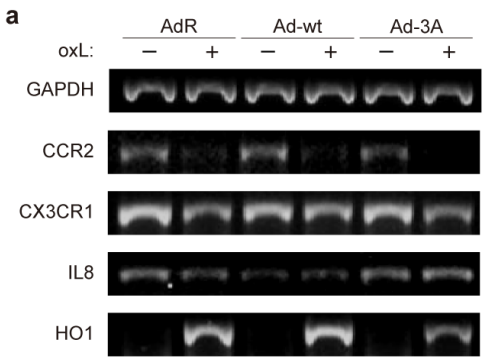
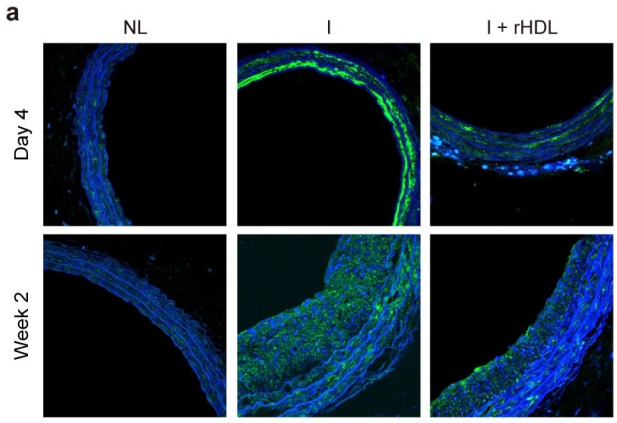
SUPPLEMENTARY FIGURES



Supplementary Fig. 1 rHDL-mediated expression of HSP27 and HO1 in lesion a. Immunohistochemistry was performed on tissue sections of balloon injured rat carotid arteries at day 4 and week 4 using an anti-HSP antibody to detect the presence of administered rHDL. Scale bar, 100 μm.



Supplementary Fig. 2 Effect of HSP27 phosphorylation status on CCRs and IL8 expression. a. Constructs were prepared adenoviral vector alone (AdR), adenoviral with wild type HSP27 (Ad-wt), and adenoviral vector with 3 Ser residue to Ala in three well-known phosphorylation sites in HSP27 (Ad-3A). THP1 cells were infected with three viral constructs for 24 hr and treated with oxidized LDL (oxLDL) for 16 hr. Then, the expression of CCR2, CX3CR1, IL8 and HO1 were analyzed by RT-PCR.



Supplementary Fig. 3 HIF1α expression in balloon injured rat carotid arteries a. The sections of balloon injured rat carotid arteries from normal group and rHDL treated group were stained with anti-HIF1α antibody to observe the relationship with hypoxia. The images were obtained by confocal microscopy (CLSM700, Leica). Nuclei were shown in blue color by DAPI staining.