The effect of SOCS1 silencing by RNA interference on apoptosis in human umbilical vein endothelial cells

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Purpose It is well known that apoptosis of endothelial cell (EC) involves the development of atherosclerosis. Down-regulation of SOCS1 (Suppressor of cytokine signalling-1) could induce apoptosis of a variety of cells. However, it has not been reported whether there is any similar phenomenon in EC. Here, we investigated the effect of SOCS1 silencing on Human Umbilical Vein Endothelial Cell (HUVEC) apoptosis induced by hypoxia/reoxygenation and its association with atherosclerosis.

Methods 1. SOCS1 expression in Human Umbilical Vein Endothelial Cell (HUVEC) was determined by RT-PCR and Western Blot. 2. Four different pairs of siRNA (siRNA-1, siRNA-2, siRNA-3, siRNA-4) were designed. 3. The negative control siRNAs with fluorescence were divided into four groups according to different concentrations (25 nmol/l, 50 nmol/l, 75 nmol/l, 100 nmol/l) and transfected into HUVEC with liposome. Fluorescent microscopy was employed to determine the concentration at which siRNAs were transfected most effectively. 4. HUVEC were divided into eight groups: four groups transfected by the different siRNAs already designed, GAPDH positive control group (GAPDH-siRNA-PC), negative control group (siRNA-NC), mock control group (Mock) and blank control group (Blank). These siRNAs were transfected at the optimal concentration. After 48 h, the one that most extremely silenced SOCS1 was selected by RT-PCR and Western Blot. 5. The selected siRNA as well as siRNA-NC was transfected at the optimal concentration. After 24 h, HUVEC were divided into four groups: a. siRNA-NC; b. siRNA-NC+hypoxia/reoxygenation; c. SOCS1 siRNA; d. SOCS1 siRNA+hypoxia/reoxygenation. Then, the expressions of Caspase-3 and Bax were detected by Western Blot, and the apoptosis rates were assessed by flow cytometry.

Results 1. It was 50 nmol/l at which siRNAs were transfected most effectively. 2. The expressions of SOCS1 in siRNA-1, siRNA-2, siRNA-3 and siRNA-4 groups declined compared to four control groups (p<0.05). siRNA-1 had the optimal silencing efficiency in four different pairs of siRNA (p<0.05). 3. After RNAi and hypoxia/reoxygenation, the expressions of Caspase-3 and Bax in SOCS1 siRNA+hypoxia/reoxygenation group increased compared to other three groups (p<0.05). The expressions of Caspase-3 and Bax in siRNA-NC+hypoxia/reoxygenation group increased compared to SOCS1 siRNA group (p<0.05). There was no statistical significance of Caspase-3 between siRNA-NG and SOCS1 siRNA groups (p>0.05). Bax in SOCS1 siRNA group was higher than that in siRNA-NC group (p<0.05). 4. After RNAi and hypoxia/reoxygenation, the apoptosis rate of SOCS1 siRNA+hypoxia/reoxygenation group increased compared to other three groups (p<0.05). The apoptosis rate of siRNA-NC+hypoxia/reoxygenation group increased compared to siRNA-NC and SOCS1 siRNA groups (p>0.05). There was no statistical significance between siRNA-NC and SOCS1 siRNA groups (p>0.05).

Conclusion 1. The expression of SOCS1 in EC could be inhibited effectively by RNAi. 2. SOCS1 silencing could exacerbate EC apoptosis induced by hypoxia/reoxygenation.