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VCAM-1 PROMOTED BY LEPTIN COULD BE OFFSET BY ADIPONECTIN THROUGH THE ACTIVATION OF AMPK VIA ADIPOR1 RECEPTOR OF VECS IN 3D VESSEL MODEL

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Objective Observe whether there exists directly antagonistic effects of Adiponectin against Leptin in vascular cells and discover which types of receptor of HUVECs and the signalling pathways work in this process.

Methods HUASMCs were suspended by I collagen and cultured in Transwell chambers. HUVECs were seeded on the surface of the collagen to finally construct a novel three-dimensional co-culture model. Then the 3D blood vessel models were divided into four groups (Leptin group, Leptin+Adiponectin group, Leptin+Losartan group and control group). Secretion of VEGF, t-PA, NO and IL-8 from HUVECs and secretion of VCAM-1, MMP-2 and MCP-1 from HUASMCs were detected by Elisa. The level of gene transcription was measured by Real-time PCR. Expression of AdipoR1 (adiponectin receptor) or AdipoR2 of HUVECs was inhibited using RNAi technology. Changes of intracellular Ca^{2+} in HUVEC were detected by Fluorescent probe Fluo-3Am. Changes of p-AMPK/AMPK, p-STAT3/STAT3 were tested by Western Blot. Compound C, PD98059, Okadaic acid and SB202190 were used to administer the antagonism by Adiponectin.

Results Secretion of VEGF and NO was increased by Leptin while t-PA was reduced and IL-8 was unaffected. Adding Adiponectin could increase those of t-PA and IL-8 and have no effect on NO and VEGF; adding Losartan had no effect on NO, VEGF and IL-8 while increased that of t-PA ($p<0.01$). Leptin increased VCAM-1, MMP-2 and MCP-1. Adding Adiponectin reduced VCAM-1 and adding Losartan reduced MMP-2 and MCP-1 ($p<0.01$). Results of Real-time PCR were the same. Receptors of HUVECs could be effectively inhibit by AdipoR1-siRNA and AdipoR2-siRNA with inhibition rates of $71.83\pm1.45\%$ and $74.89\pm1.12\%$ ($p<0.01$). The effect of Adiponectin in increasing IL-8 and reducing VCAM-1 was diminished by the depression of AdipoR1. Adiponectin increased the concentration of intracellular Ca^{2+} of HUVECs. Western Blot showed that Leptin increased the expression of p-STAT3 and Adiponectin increased p-AMPK. The effect of Adiponectin in increasing IL-8 and reducing VCAM-1 could be inhibited by Compound C. The effect of Adiponectin in reducing VCAM-1 was partially inhibited by PD98059.

Conclusion Cell growth and secretion of bioactive substances in 3D co-culture model were different from the plane culture. Crosstalk between HUVECs and HUASMCs was mediated by IL-8 and it reduced secretion and transcription of VCAM-1 via ERK pathway. By activating AdipoR1, Adiponectin increased the concentration of intracellular Ca^{2+} , and exerted partial anti-inflammatory effects against Leptin though the AMPK pathway. Adiponectin might also increase the secretion of IL-8 in HUVECs to reduce VCAM-1 from HUASMCs.