**Abstract: P363**

**Nucleostemin is prerequisite for Notch signaling and pro-inflammatory phenotype conversion of the endothelial cell in atherosclerosis**

**Authors:**

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**Topic(s):**

Atherosclerosis, Cerebrovascular Diseases, Aneurysm, Restenosis

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**Introduction/ Background**

Endothelial cell (EC) dysfunction by pro-inflammatory phenotype conversion (Pro-Inf) is a crucial step in atherosclerosis. Notch, a signaling of angiogenesis, is reactivated in EC of the advanced lesion of atherosclerosis. The nucleolar GTP-binding protein nucleostemin (NS) maintains the stemness of the various type of stem cells in the embryonic stage and is down-regulated in adult, but is again up-regulated in the cancer cells. We focused attention to NS as the key regulator of inflammation and proliferation of EC in atherosclerosis.

**Purpose**

This study first investigated the role of NS in Notch signaling and Pro-Inf of EC.

**Methods**

Enzyme-immunohistochemistry was performed in the formalin-fixed coronary arteries of the autopsied human samples. Human arterial endothelial cells were cultured and NS mRNA was knocked-down (NS-KD) by specific siRNA. Immunofluorescence, ELISA, real time-PCR and western blotting were performed in order to investigate the gene and protein expression. The round slices of the rat thoracic aorta was explanted and cultured for angiogenesis assay. Apoptosis was assayed by the treatment with doxorubicin.

**Results**

Intense immune-reactivity of NS was observed in the luminal EC and intimal neo-vascularization of human atherosclerotic lesions. These EC also had the immune-reactivity not only of Notch ligands (Jagged1, DLL4), receptors (Notch1) and target (Hes1), but also of the mediators for Pro-Inf (IL1 receptor, VCAM1). NS-KD in the EC inhibited the nuclear translocation of activated Notch1 intracellular domain (-85%: immunofluorescence) (Figure). Moreover, NS-KD inhibited the proliferation of EC (-15%) and the endothelial sprouting from the rat aortic ring explant. Thus, NS is prerequisite for Notch 1 signaling. NS-KD suppressed the production of IL-6 (-71%) and IL-8 (-80%) (ELISA) and suppressed the up-regulation of VCAM1 and E-selectin induced by IL-1 alpha (RT-PCR, western blotting), indicating NS is necessary for Pro-Inf. But on the contrary, NS-KD significantly down-regulated endothelial nitric oxidase and heme oxygenase-1. It enhanced cell apoptosis induced by doxorubicin with increase in the production of cleaved caspase 3, indicating NS supports anti-inflammatory systems.

**Conclusion**

NS is prerequisite for the progression of atherosclerosis by maintaining proliferation, inflammation and survival of EC.
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