**Nuclear endothelial nitric oxide synthase interacts with RNA-binding proteins to modulate endothelial gene expression.**

Xiaozhu Zhou[1,2], Ilka Wittig[2,3], Mauro Siragusa[1,2], Ingrid Fleming[1,2]

[1]Institute for Vascular Signalling, Centre for Molecular Medicine, Goethe University, Frankfurt am Main, Germany.

[2]German Center for Cardiovascular Research (DZHK), Partner site RheinMain, Frankfurt am Main, Germany.

[3]Functional Proteomics, SFB 815 Core Unit, Faculty of Medicine, Goethe University, Frankfurt am Main, Germany.

**Background and Aim.** Endothelial nitric oxide (NO) synthase (eNOS) is responsible for the production of NO, an autacoid that regulates vascular tone and endothelial cell homeostasis. The effect of NO can be attributed to its interaction with heme-containing proteins, such as soluble guanylyl cyclase, or to its reaction with cysteines, a process referred to as *S*-nitrosation. Several studies have reported that eNOS can be detected in the nucleus and this study set out to determine its nuclear interaction partners.

**Methods and Results.** Stimulation of primary human endothelial cells with vascular endothelial growth factor (VEGF; 50 ng/ml, 10 minutes) induced the nuclear translocation of eNOS. Once in the nucleus eNOS associated with 81 proteins (immunoprecipitation and proteomics), most of which have been reported to be *S*-nitrosated. One such protein was double-stranded RNA-specific adenosine deaminase (ADAR1), an enzyme involved in the deamination of adenosine to inosine in double-stranded RNA (dsRNA), leading to decreased dsRNA stability. Interestingly, the knockdown of eNOS in primary endothelial cells (shRNA) was associated with an increase in dsRNA levels (immunofluorescence). Moreover, the overexpression of the wild-type eNOS or a gain-of-function mutant (eNOS-Y657F) in HEK cells decreased dsRNA content, which was elevated in cells expressing a catalytically inactive eNOS mutant (eNOS-Y657D). Nuclear eNOS was also associated with core components of paraspeckles, specialized nuclear compartments involved in the regulation of gene expression. Indeed, the shRNA-mediated knockdown of eNOS in primary human endothelial cells profoundly affected the expression of >4k genes.

**Conclusions.** These results demonstrate that eNOS and NO signalling (*S*-nitrosation) modulate nuclear processes that are essential for the regulation of endothelial gene expression.

**Keywords**: eNOS, ADAR, paraspeckles