CRUCIAL ROLE OF ARYL HYDROCARBON RECEPTOR IN INDOXYL SULFATE-INDUCED VASCULAR INFLAMMATION

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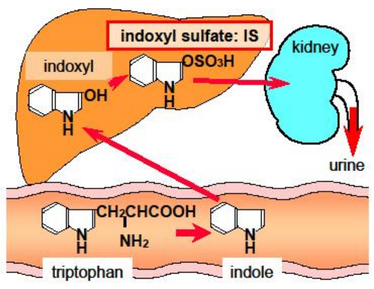
Backgrounds

- **Chronic kidney disease (CKD) is an independent risk factor for cardiovascular disease (CVD)**

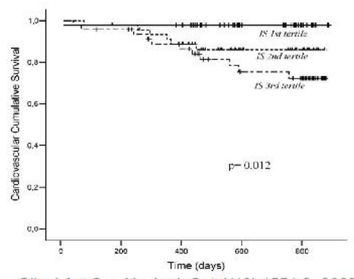
(N Engl J Med; 351:1296-1305., 2004)

- **Indoxyl sulfate (IS) is associated with vascular disease and mortality in CKD patients**

(Clin J Am Soc Nephrol; Oct;4(10):1551-8., 2009)



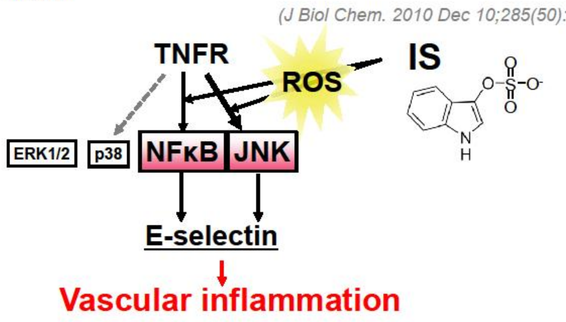
Am J Nephrol; 14(3):207-12. 1994



Clin J Am Soc Nephrol; Oct;4(10):1551-8. 2009

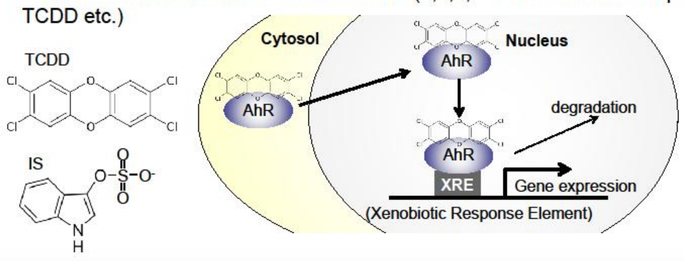
- **IS induces leukocyte-endothelial interactions through up-regulation of E-selectin**

(J Biol Chem. 2010 Dec 10;285(50):38869-75 2010)



Arylhydrocarbon Receptor (AhR)

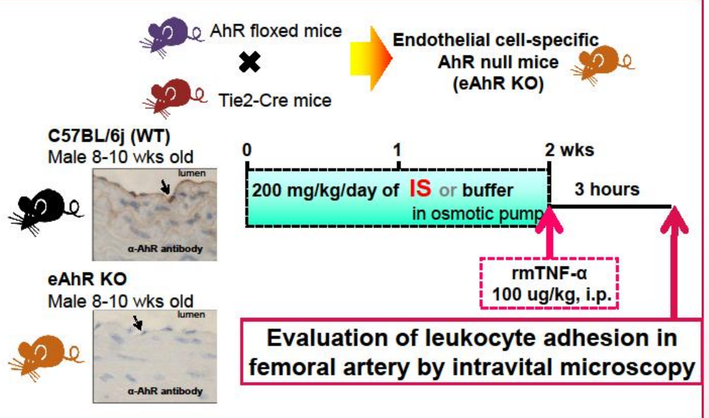
- **binds to IS** *(Biochemistry. 2010 Jan 19;49(2):393-400.)*
- a ligand-inducible transcription factor
- mediates a wide range of toxic, teratogenic, and carcinogenic effects of environmental contaminants such as dioxin (2,3,7,8-Tetrachlorodibenzo-p-dioxin: TCDD etc.)



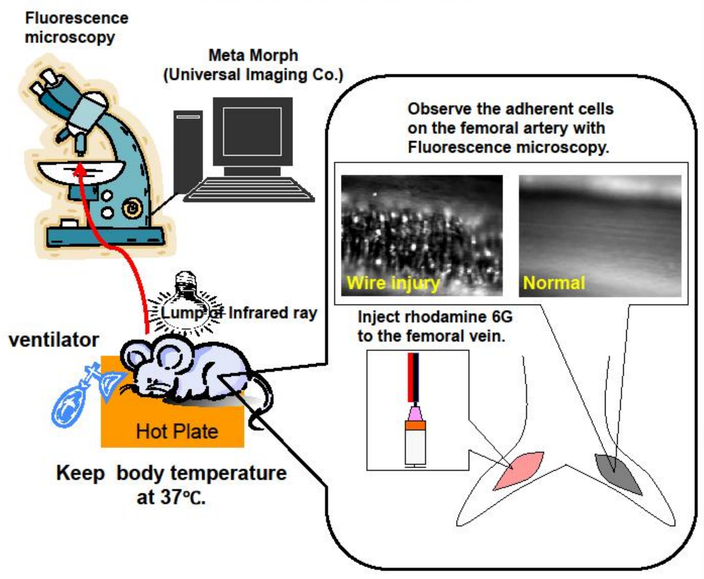
Objective

To examine the role of AhR in IS-induced leukocyte-endothelial interaction

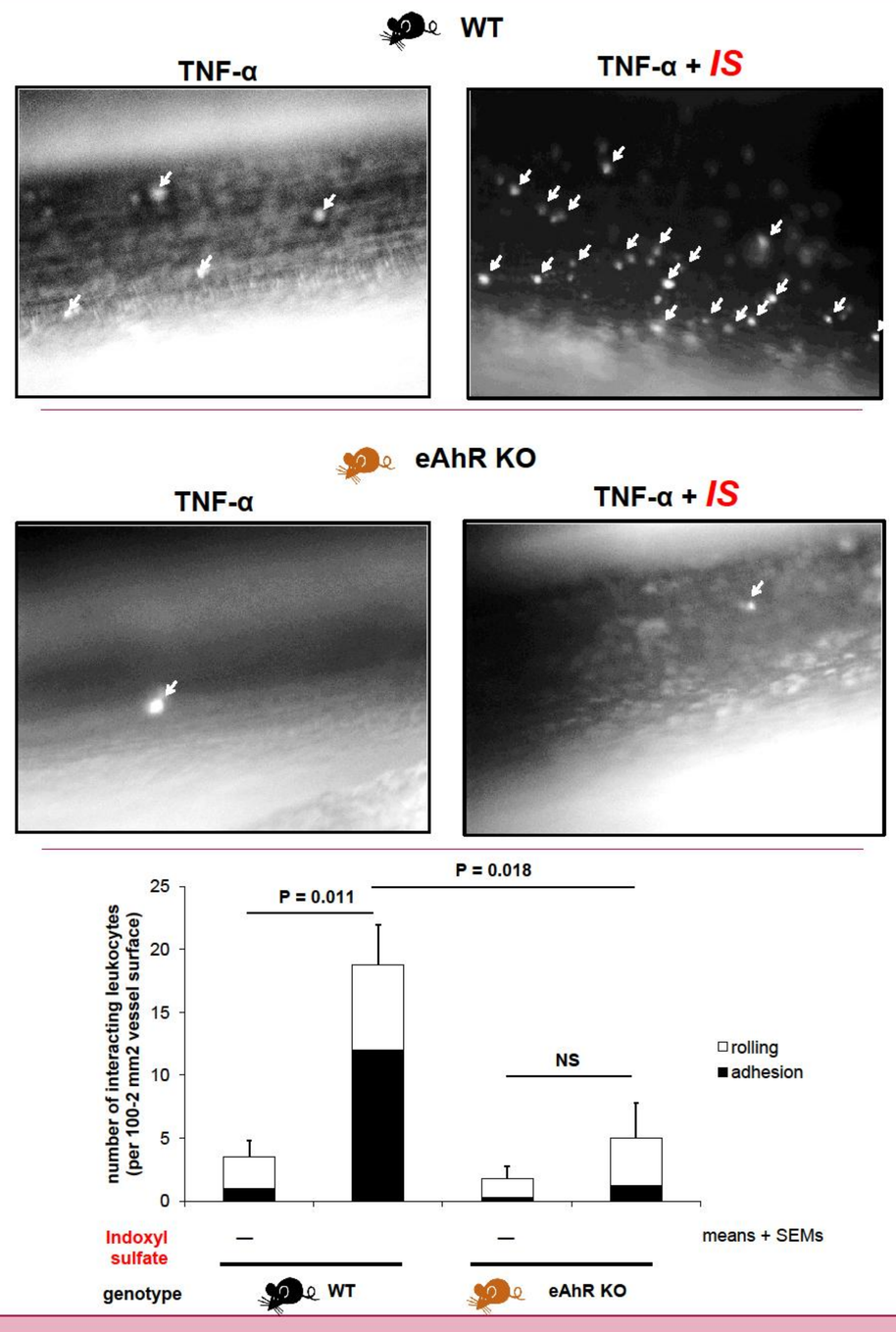
Methods: *in vivo*



Intravital Microscopy



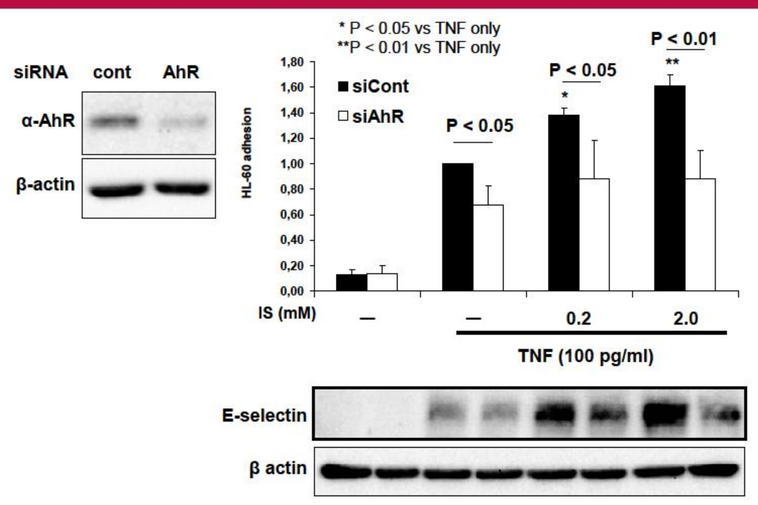
Results 1: *in vivo*



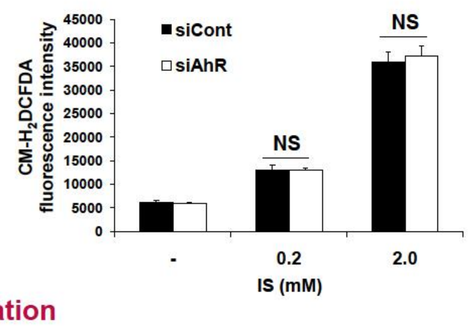
Methods: *in vitro*

- **Cells**: Human umbilical vein endothelial cells (HUVEC) transfected with siRNA of AhR (siAhR) or control (siCont)
- **Stimulation**: 0.2, or 2.0 mmol/L of IS for 20 hours, + 100 pg/ml of TNF-α for 4 hours
- **Leukocyte adhesion**: HL-60 cells (human leukemia cell line)
- **ROS detection**: CM-H_2DCFDA
- **Transcriptional activity**: Luciferase assay

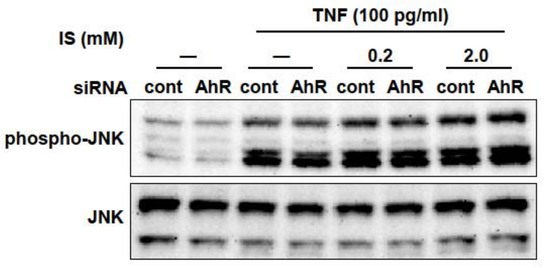
Results 2: *in vitro*



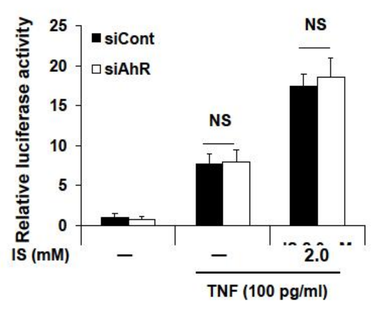
ROS production



JNK phosphorylation

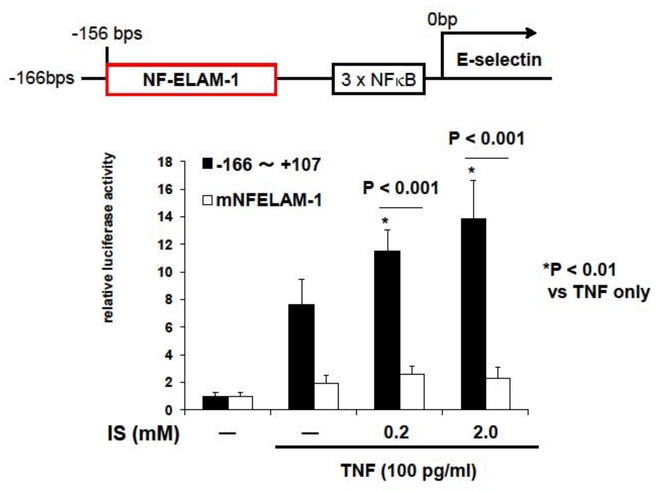


NFκB activation

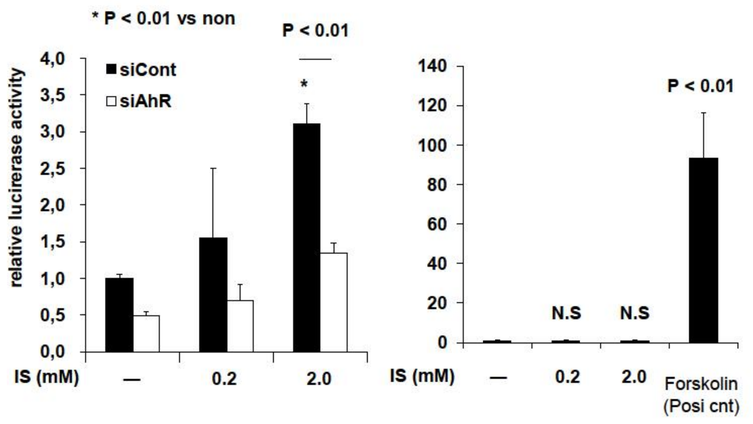


Results 3 : *in vitro-2*

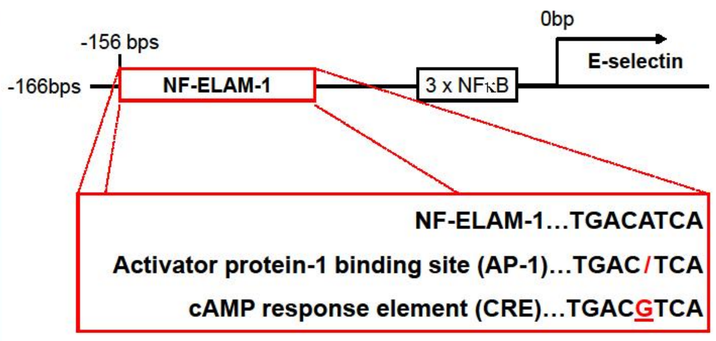
Luciferase assay using E-selectin promoter



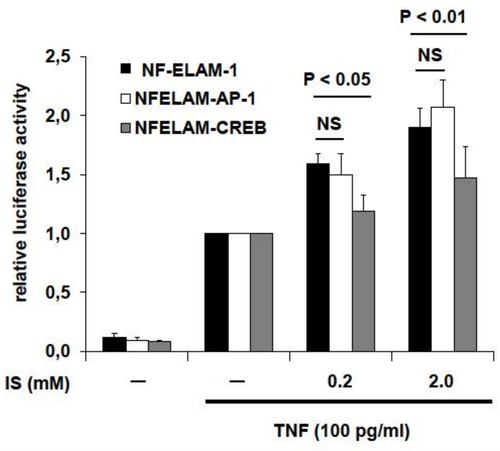
Luciferase assay



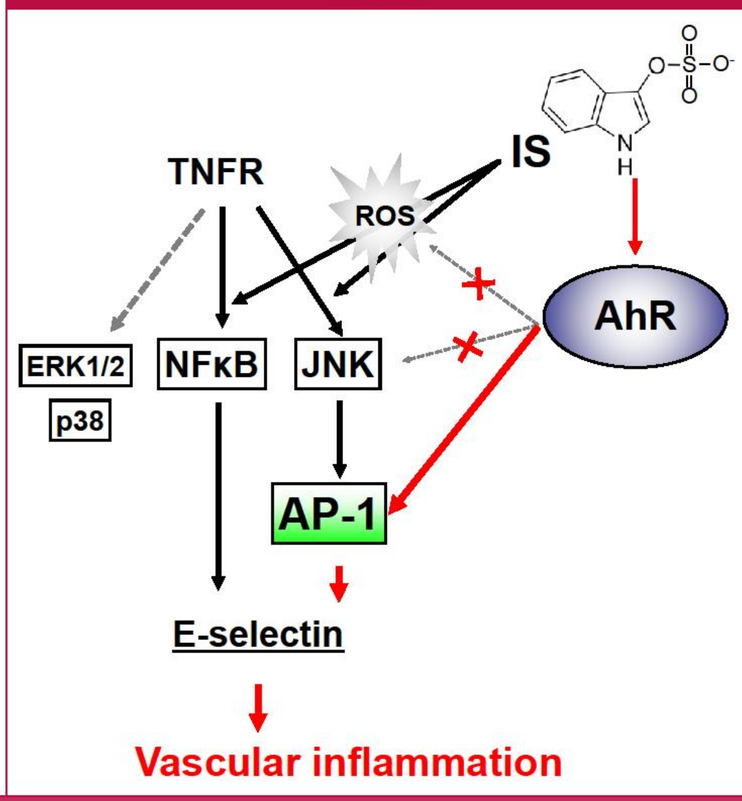
The detailed sequence of NF-ELAM-1 site



Luciferase assay using NFELAM-1 mutants



Model



Conclusion

Activation of AhR by indoxyl sulfate enhanced vascular inflammation and E-selectin expression through transcriptional activation of AP-1

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