

Yassine Bouatou – Journal Club du 13.02.2014 – Service de Néphrologie

**INDOLIC UREMIC SOLUTES INCREASE TISSUE FACTOR
PRODUCTION IN ENDOTHELIAL CELLS BY THE ARYL
HYDROCARBON RECEPTOR PATHWAY**

KIDNEY INT. 2013 OCT;84(4):733-44.

Background

- MRC associée de manière indépendante à une augmentation de la morbi-mortalité CV
- MRC associée à une accumulation de toxines :
 - Petites molécules (Acide guanido acétique, méthylguanidine, ...)
 - Moyennes molécules (β_2 M, ...)
 - Molécules à forte liaison protéique (homocystéine, leptine, des métabolites du tryptophane comme l'indoxyl sulfate (IS) et l'indole-3-acetic acid (IAA), ...)

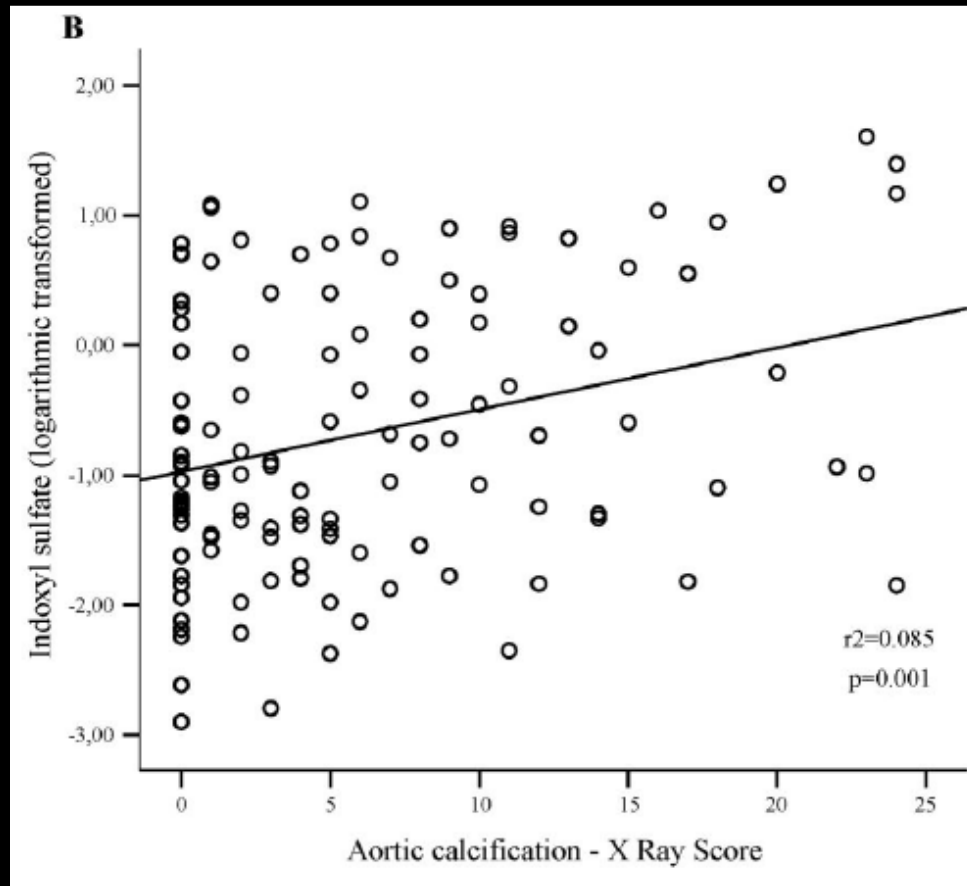
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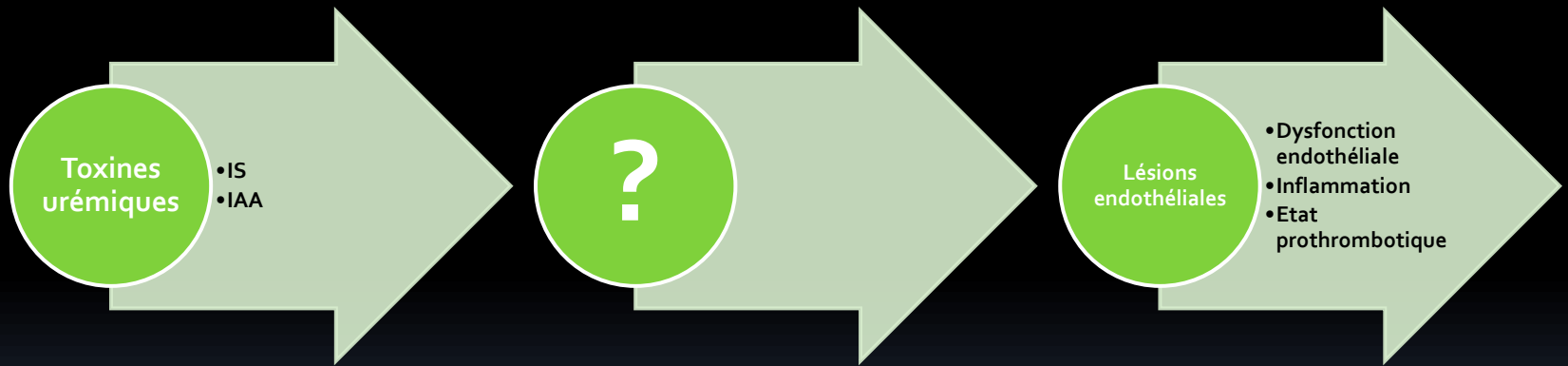
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Background – IS et calcification vasculaire



Barreto FC, Barreto DV, Liabeuf S *et al.* Serum indoxyl sulfate is associated with vascular disease and mortality in chronic kidney disease patients. *Clin J Am Soc Nephrol* 2009; 4: 1551–1558.

Background



Méthode

- Approche non *a priori* pangénomique
- Analyse par *microarrays*
- 73 patients hémodialysés
- 50 patients avec une MRC non dialysés
- Critères d'inclusion :
 - Âge > 18 ans
 - Hb > 110 g/L
 - Pas d'évènement CV récent
 - Pas d'infection récente (< 3 mois)
 - Pas d'intervention chirurgicale récente
 - En dialyse > 6 mois
 - Pas de traitement immunosuppresseur en cours
- 43 contrôles

Méthode

Table 3 | Baseline characteristics of controls, undialyzed CKD patients, and HD patients

	Controls (n = 43)	CKD (n = 50)	HD (n = 73)
Age (years)	64 (38; 78)	63 (26; 89)	68 (23; 91)
Sex ratio (F/M)	23/20	19/31	31/42
Dialysis vintage (months)	—	—	6 (0.75; 30)
Body mass index (kg/m ²)	25.12 ± 3.33	25.04 ± 4.4	24.3 ± 5.2
<i>Kidney disease</i>			
Glomerulonephritis	—	7 (14%)	17 (23%)
PKD	—	5 (10%)	5 (7%)
Vascular nephropathy	—	11 (22%)	22 (29%)
Interstitial nephropathy	—	15 (29%)	10 (13%)
Other hereditary	—	3 (6%)	2 (3%)
Unknown	—	9 (17%)	17 (22%)
SBP (mmHg)	128.9 ± 19.06	141.7 ± 23.94 ^a	144.4 ± 24.31 ^{a,b}
DBP (mmHg)	75.49 ± 10.27	81.18 ± 11.24 ^a	73.26 ± 15.87 ^b
Smokers	—	26 (51%)	27 (36%)
History of cardiovascular disease	0	16 (31%)	25 (34%)
Hypertension	2 (5%)	44 (86%)	54 (73%)
Statins	0	15 (29%)	25 (34%)
Antihypertensive agents	2 (5%)	44 (86%)	40 (54%)
Aspirin or antiaggregant agents	0	11 (22%)	38 (51%)
EPO therapy	0	11 (22%)	55 (74%)
Anticoagulant agents	0	8 (16%) ^a	16 (21%) ^{a,b}
Hemoglobin (g/dl)	14.27 ± 1.03	12.5 ± 1.8 ^a	11.66 ± 1.6 ^{a,b}
Albumin (g/l)	40 (31; 59)	37 (31; 41) ^a	35 (26; 46) ^{a,b}
Calcium (mmol/l)	2.31 (2.17; 2.52)	2.33 (2; 2.55)	2.36 (1.84; 2.66)
Phosphorus (mmol/l)	1.09 (0.74; 1.46)	1.14 (0.65; 2.20)	1.46 (0.54; 3.17) ^{a,b}
Uric acid (3 mol/l)	297 (197; 451)	438 (175; 828) ^a	342 (197; 524) ^b
Urea (mmol/l)	5.5 (3.2; 9.3)	14.8 (4.9; 44.7) ^a	19 (7.6; 39.6) ^{a,b}
Creatinine (mol/l)	69 (48; 107) ^{a,b}	209 (84; 640) ^a	783 (213; 1533) ^{a,b}

Abbreviations: CKD, chronic kidney disease; DBP, diastolic blood pressure; EPO, erythropoietin; HD, hemodialyzed; PKD, polycystic kidney disease; SBP, systolic blood pressure.

Results are given as mean ± s.d. if Gaussian distribution or in median (min.; max.) if not.

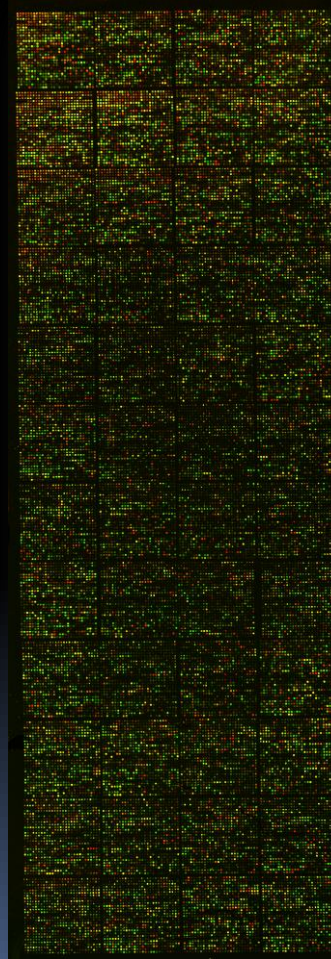
^aSignificantly different versus control.

^bSignificantly different versus CKD.

Méthode : rappel


- Pour estimer l'expression d'un gène :
 - ARN : Northern Blot quantitatif, short/long nucleotide array
 - Protéine : Western Blot quantitatif, chromatographie
- <http://www.bio.davidson.edu/courses/genomics/chip/chip.html>

Méthode : rappel





Résultats

- *Microarray* après incubation de cellules endothéliales de cellules endothéliales de veines ombilicales humaines avec de l'IS 1 mmol/l pendant 4 heures
 - IS associé à l'augmentation de l'expression de 50 gènes
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Résultats

Table 1 | Upregulated genes identified by microarray analysis after incubation of HUVECs with IS (1 mmol/l) for 4 h

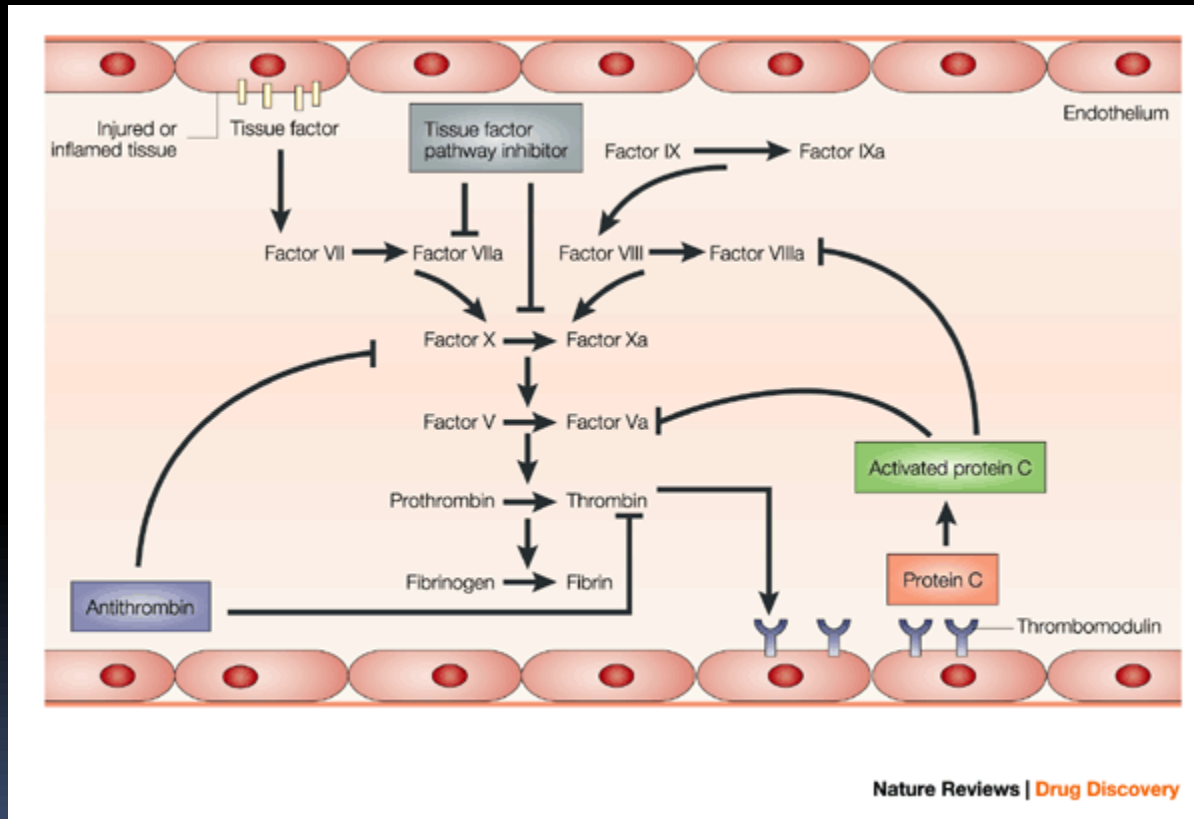
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0.002	<i>F2RL3</i>	Coagulation factor II (thrombin) receptor-like 3
0.006	<i>VIPR1</i>	Vasoactive intestinal peptide receptor 1
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0.002	<i>TIPARP</i>	TCDD-inducible poly(ADP-ribose) polymerase
0.018	<i>CYP26B1</i>	Cytochrome P450, family 26, subfamily B, polypeptide 1
0.002	<i>ITGA11</i>	Integrin, α 11
0.002	<i>APOLD1</i>	Apolipoprotein L domain containing 1, transcript variant 2
0.004	<i>SECTM1</i>	Secreted and transmembrane 1
0.002	<i>F3</i>	Coagulation factor III (thromboplastin, tissue factor)
0.009	<i>PTGS2</i>	Prostaglandin G/H synthase and cyclooxygenase
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0.035	<i>SAMD12</i>	Sterile α -motif domain containing 12, transcript variant 1
0.025	<i>POU4F3</i>	POU class 4 homeobox 3
0.004	<i>BMF</i>	Bcl2 modifying factor, transcript variant 1
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0.048	<i>SLC16A6</i>	Solute carrier family 16, member 6
0.009	<i>LIF</i>	Leukemia inhibitory factor (cholinergic differentiation factor)
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0.009	<i>PRKG1</i>	Human mRNA for type I β -cGMP-dependent protein kinase

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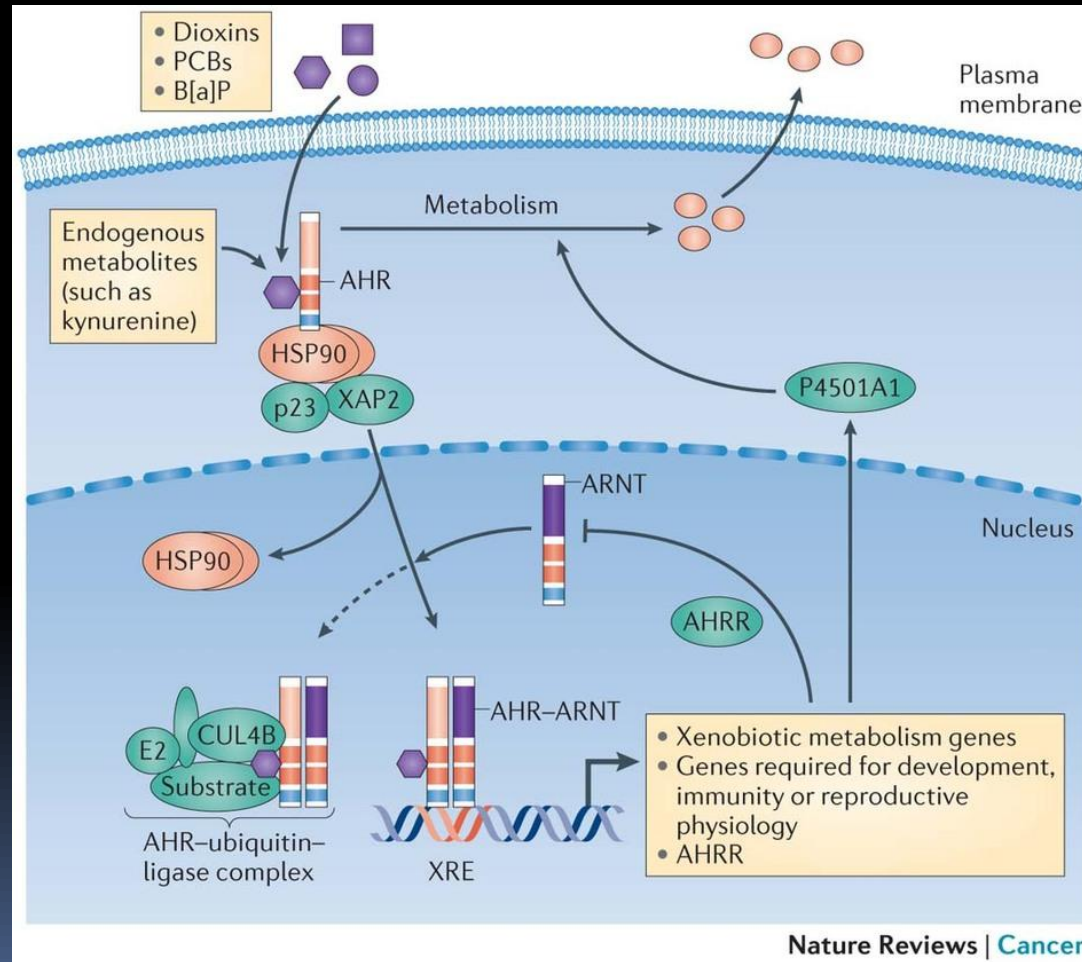


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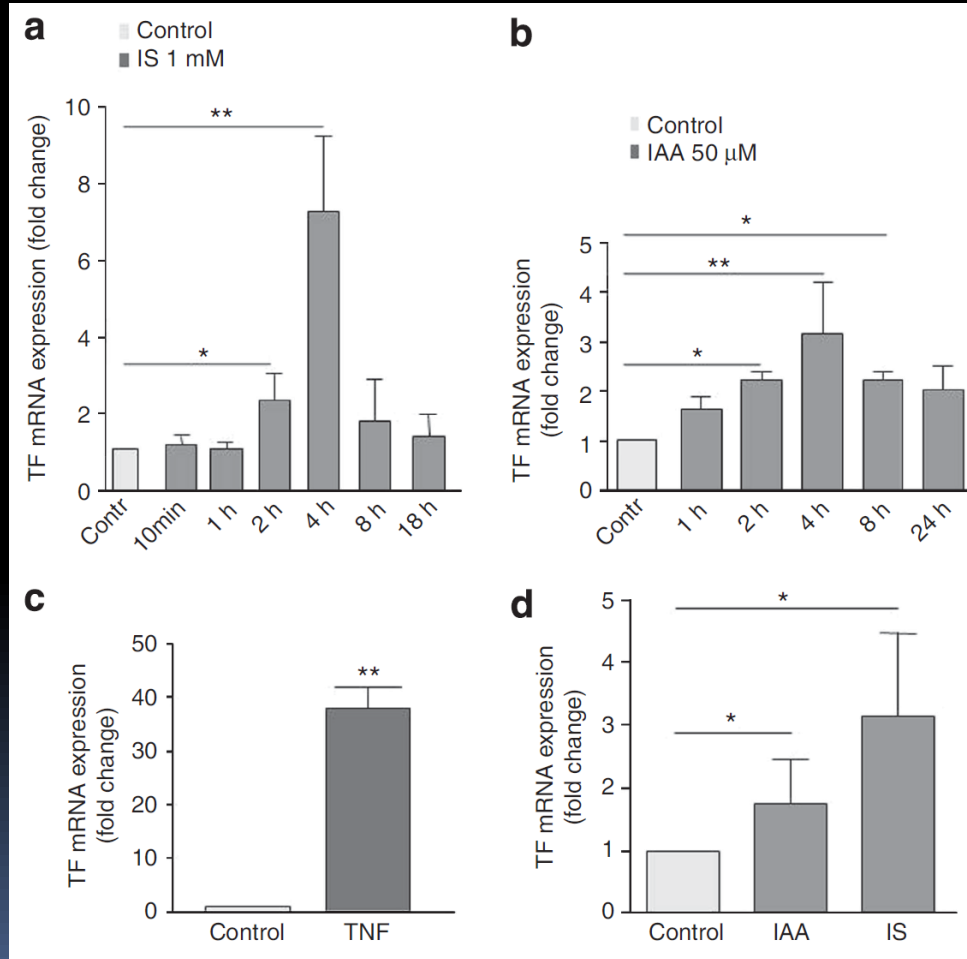
Résultats

Table 2 | Upregulated genes identified by RT-PCR after incubation of HUVECs with IS (1 mmol/l) or IAA (50 μ mol/l) for 4 h

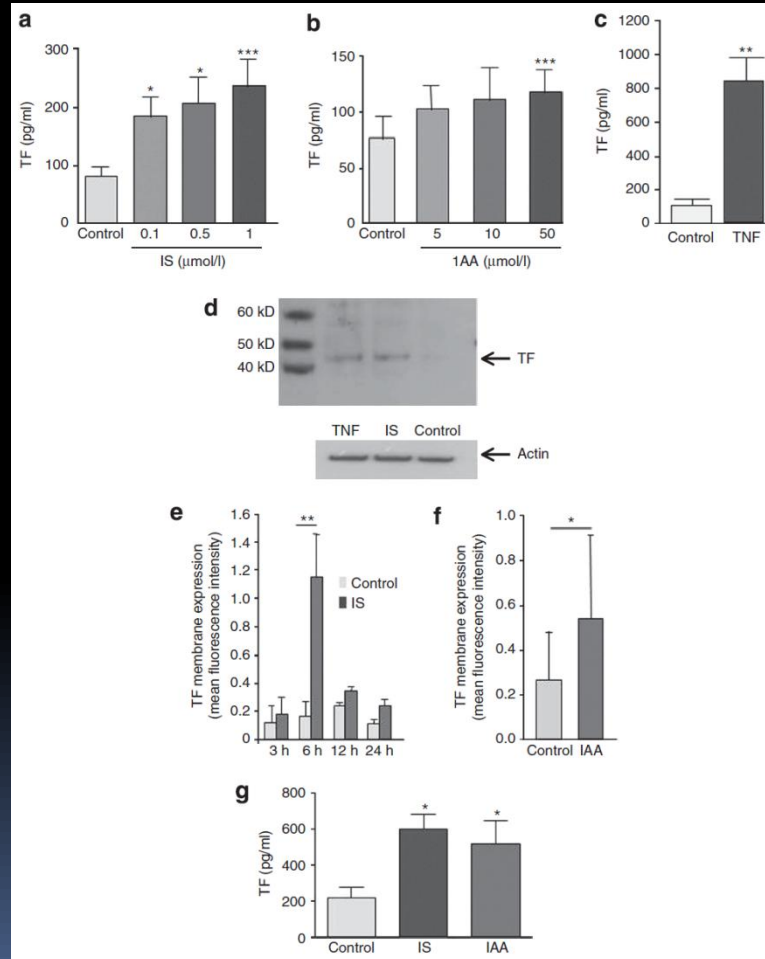
Gene	Fold change of expression after incubation with IS	Fold change of expression after incubation with IAA
<i>CYP1B1</i>	200.1 \pm 91.5	101.8 \pm 30.3
<i>CYP1A1</i>	21.4 \pm 7.9	14.9 \pm 5.2
<i>CYP1A2</i>	4.1 \pm 2.0	4.0 \pm 0.6
<i>AHRR</i>	7.3 \pm 1.9	4.5 \pm 1.0
<i>CCR7</i>	6.3 \pm 4.2	3.1 \pm 0.7
<i>TIPARP</i>	6.1 \pm 5.5	2.8 \pm 0.2
<i>PTGS2</i>	3.7 \pm 1.2	2.2 \pm 0.4
<i>TGFB3</i>	2.1 \pm 0.9	1.8 \pm 0.1

Abbreviations: HUVECs, human umbilical vein endothelial cells; IAA, indole-3-acetic acid; IS, indoxyl sulfate; RT-PCR, reverse transcriptase PCR.

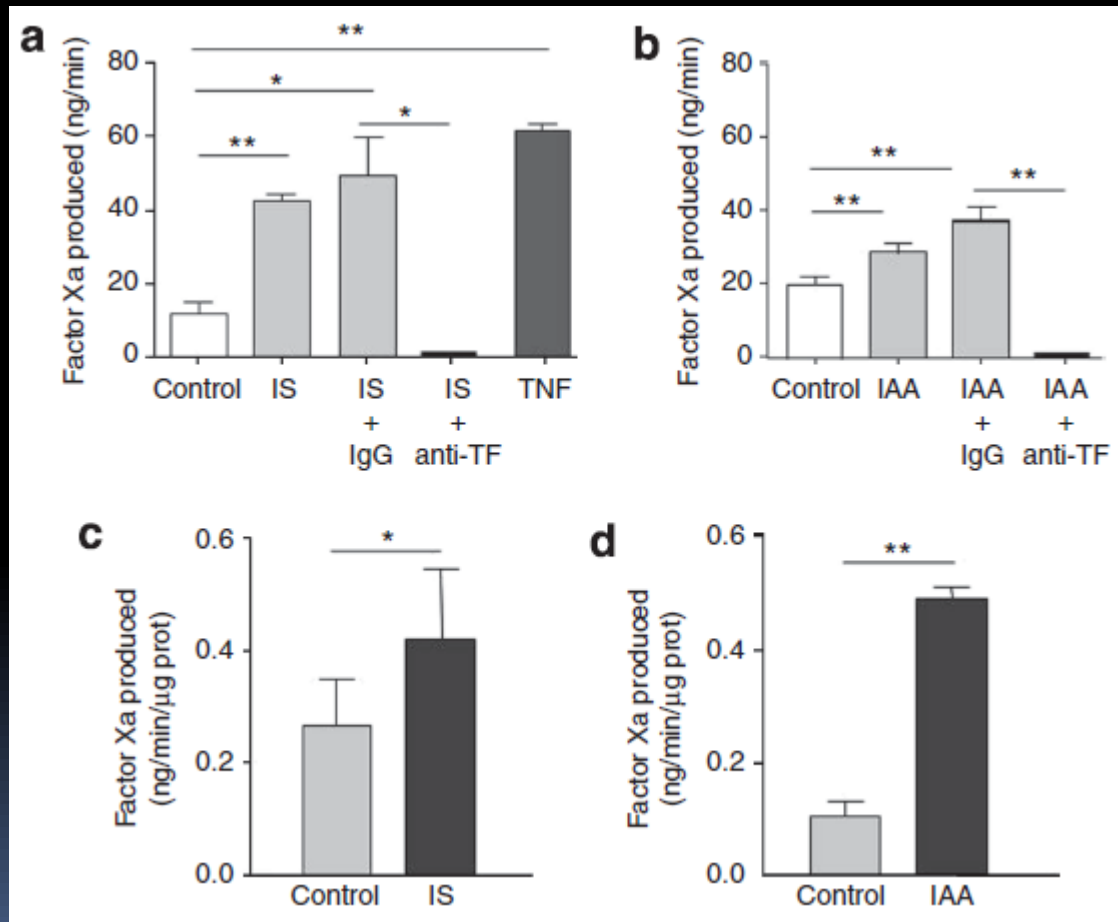
Les solutés indolés augmentent l'expression du gène *F3*



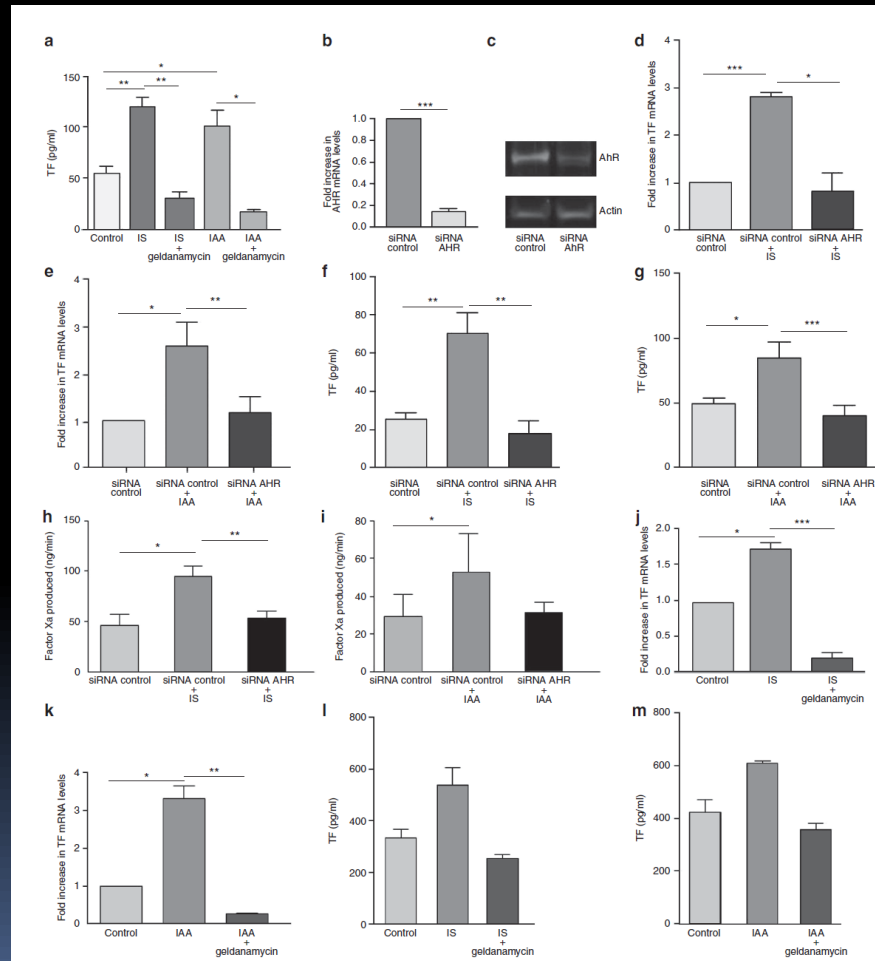
Les solutés indolés augmentent la production de TF in vitro



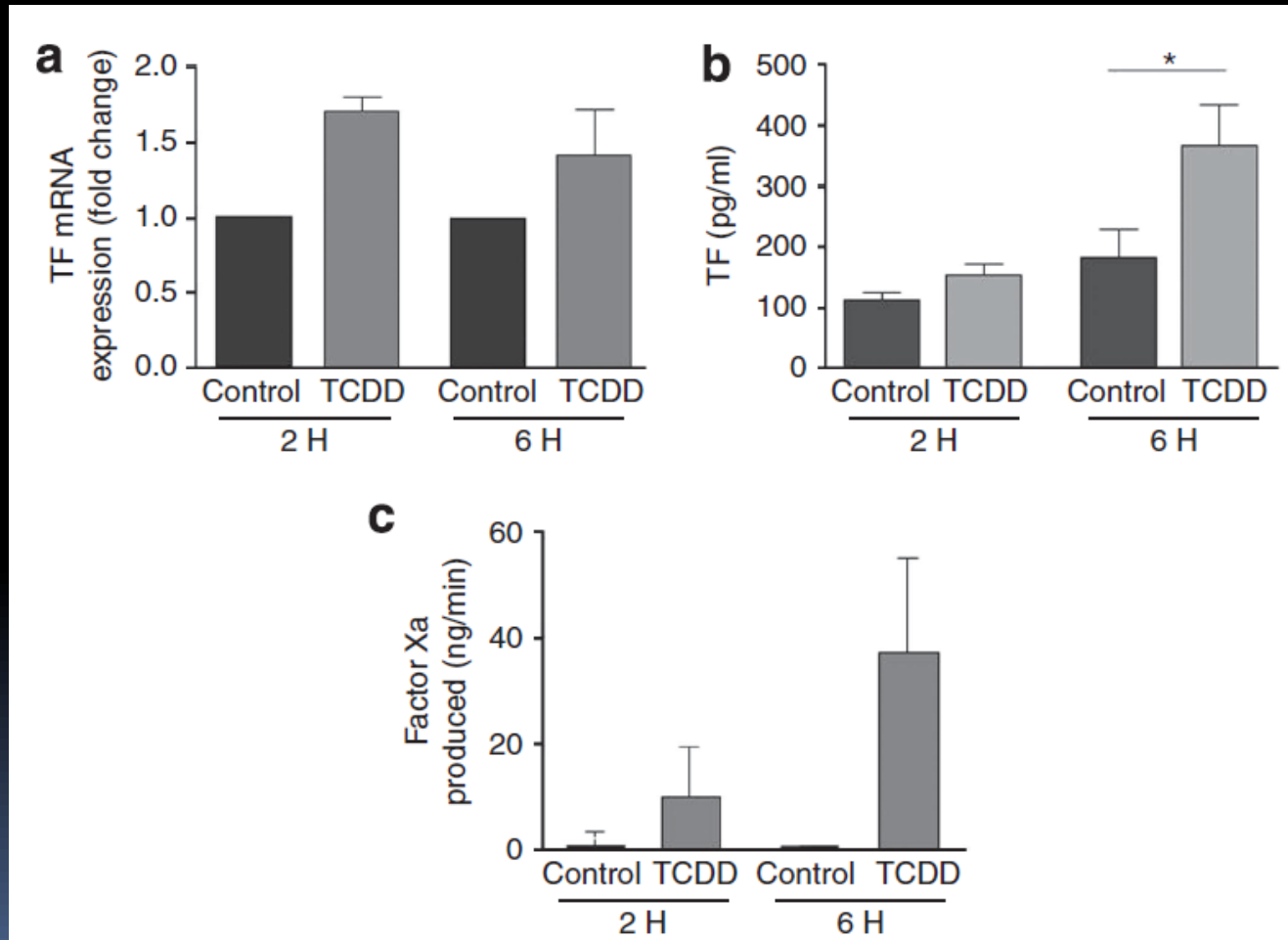
Les solutés indolés augmentent l'activité pro coagulante de TF dans les cellules endothéliales et les microparticules endothéliales



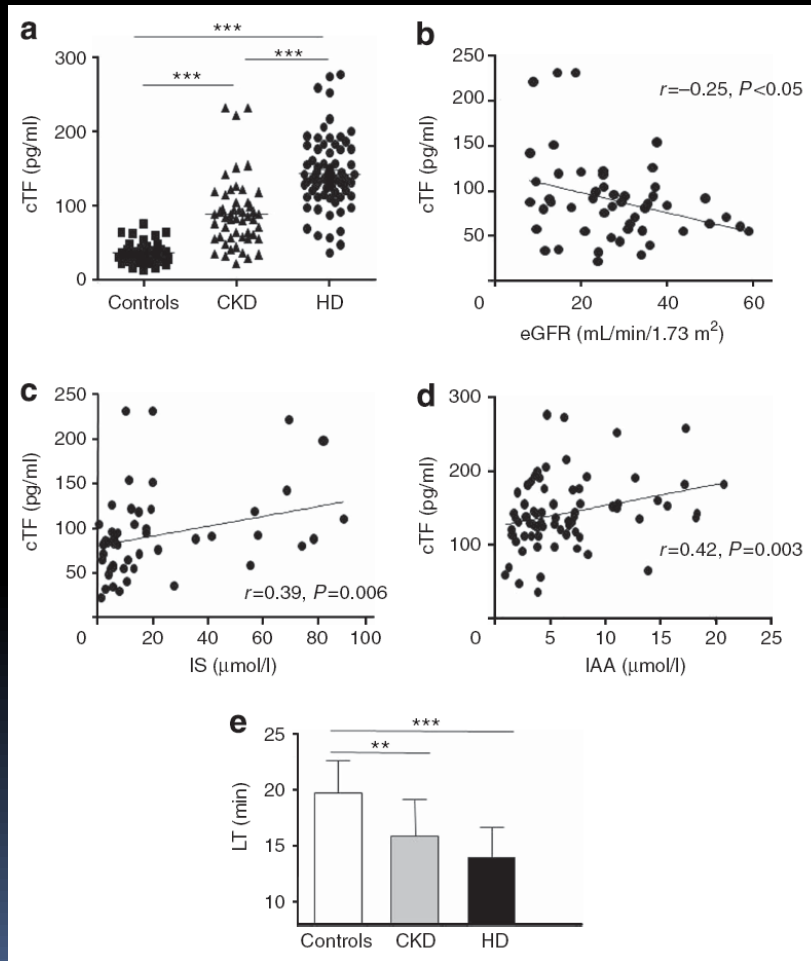
Implication de la voie AHR dans la production de TF par les cellules endothéliales



Confirmation TCDD



Résultats



Discussion

- Solutés indolés modulent la production de TF via l'AHR.
- IS et IAA sont des ligands récemment décrits de l'AHR
- Autre ligand connu de l'AHR : TCDD (Dioxine)
- Inhibition de la production de TF par l'inhibition de Nfκ-B est incomplète

Discussion – Perspectives

- Autre ligand de l'AHR et toxine urémique : acide kynurenique.

Nature Volume: 478, Pages: 197–203.

- Concentrations toxiques d'IAA et d'IS restent à déterminer *in vivo*.
- Certaines toxines urémiques pourraient avoir un effet « *dioxin like* ».

Discussion – Perspectives

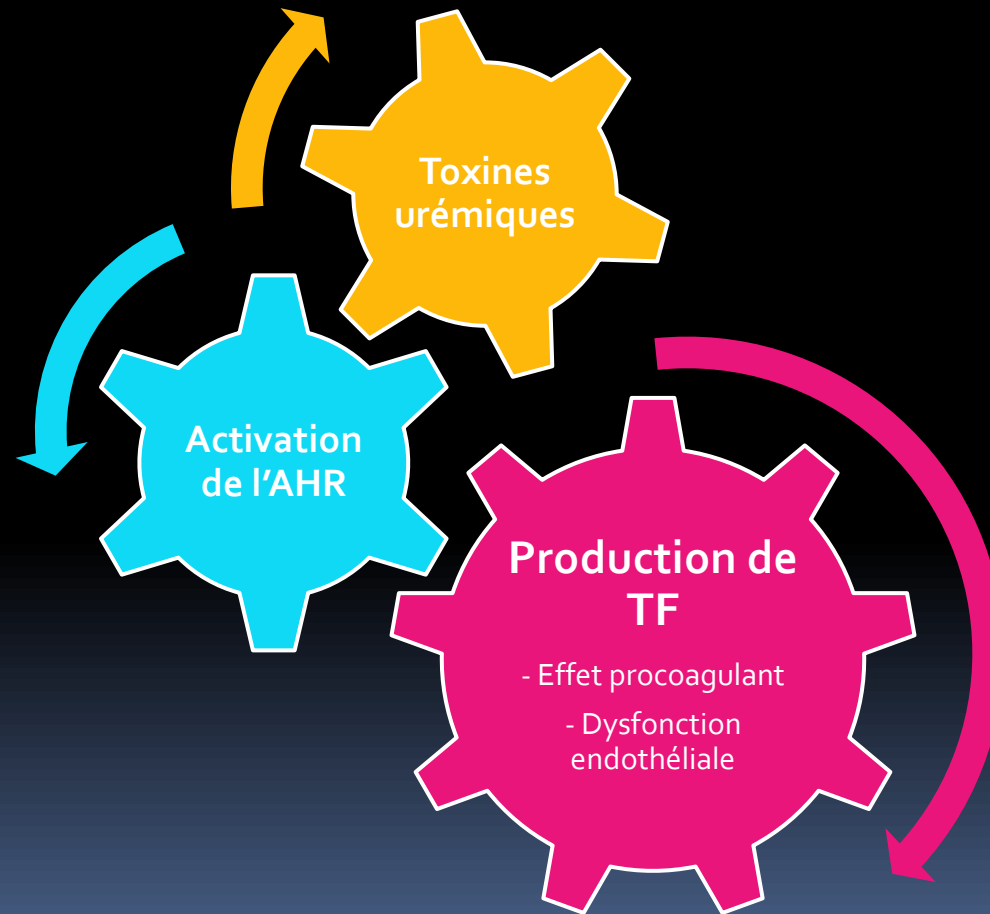
- Effets d'une activation de l'AHR rapportés dans la littérature
 - **“Chef d'orchestre” de la toxicité à la dioxine**
Groupe de Saurat JH, Sorg O, Samer CF, Swiss Center for Applied Human Toxicology (SCAHT)
 - **Effet sur la différenciation en Th17 et Treg**
Nature. 2008 May 1;453(7191):65-71.
 - **Effet direct sur l'expansion et la différenciation des cellules hématopoïétiques progénitrices.**
Blood. 2013 Jul 18;122(3):376-85.
 - **Effet sur la différentiation et la progression tumorale.**
Nature. 2011 Oct 5;478(7368):197-203.
 - **Nouvelle cible d'immunomodulation en transplantation?**
Transplantation. 2013 Apr 27;95(8):983-90.

Discussion – Limites

- Surestimation *in vitro* versus *in vivo*.



Conclusion



Merci 😊

