Modulation of anti-aging gene Klotho (KL) in patients with Delayed Graft Function (DGF) and Ischemia/Reperfusion (I/R) Injury: possible role of Complement in the regulation of transplant cellular senescence

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Introduction: The KL aging suppressor gene encodes a trans-membrane protein that is mainly produced at renal level and is released into blood as an endocrine anti-aging factor. Recent studies demonstrated a reduction of renal and soluble KL in acute kidney injury.

Aim: To investigate the modulation of KL in a swine model of I/R injury and in patients affected by DGF.

Methods: In an experimental model of I/R injury, 10 pigs underwent to 30 min of renal warm ischemia followed by 24h of reperfusion. To investigate the role of Complement in this setting, recombinant C1-inhibitor (C1-Inh) was administered in 5 pigs 5 min before the start of reperfusion. Renal KL expression was investigated by immunohistochemical analysis in renal biopsies from animal model and transplant patients. ELISA assay was assessed to determine soluble KL in transplant patients sera. We also evaluated KL *in vitro* in renal tubular epithelial cells (HK-2) stimulated with C3a by western blot.

Results: By immunohistochemical analysis (**Fig1**) we found that I/R injury induced a significant reduction in tissue KL at 24h from reperfusion compared to basal condition (T24 Ctr:30.4% 1.2% vs. T0:72.5% 2.1%, p<0.05). Complement inhibition significantly preserved KL protein expression in renal tubular epithelial cell *in vivo* (T24 C1-inh: 70.7% 1.9% vs. T24 Ctr, p<0.05). In accordance, activation of renal HK-2 cells by Complement anaphylotoxin C3a in vitro (10^{-7}M for 24h) led to significant downregulation of KL protein as showed by Western Blot analysis (**Fig2**). When we analyzed biopsies from patients with DGF, we found a significant reduction in tubular KL compared to pre-transplant (Pre-Tx) expression levels (DGF:10.8% 0.6% vs. pre-Tx:73.1% 2.5%, p<0.05) (**Fig3**). Finally, when we compared DGF patients after 2 years from transplantation with early graft function (EGF) patients with similar renal function, we found a significant difference in KL serum levels (DGF: 412pg/ml 106 vs. EGF:900.25pg/ml 153.9,p=0.03) by ELISA assay (**Fig3**).

Conclusions: Our study showed for the first time that the Complement system is pivotal in regulating KL production in tubular epithelial cells in I/R injury. Considering the central role of KL in preventing cellular senescence and apoptosis, we hypothesize that local and systemic KL deficiency might play a central role in DGF-associated chronic allograft dysfunction.

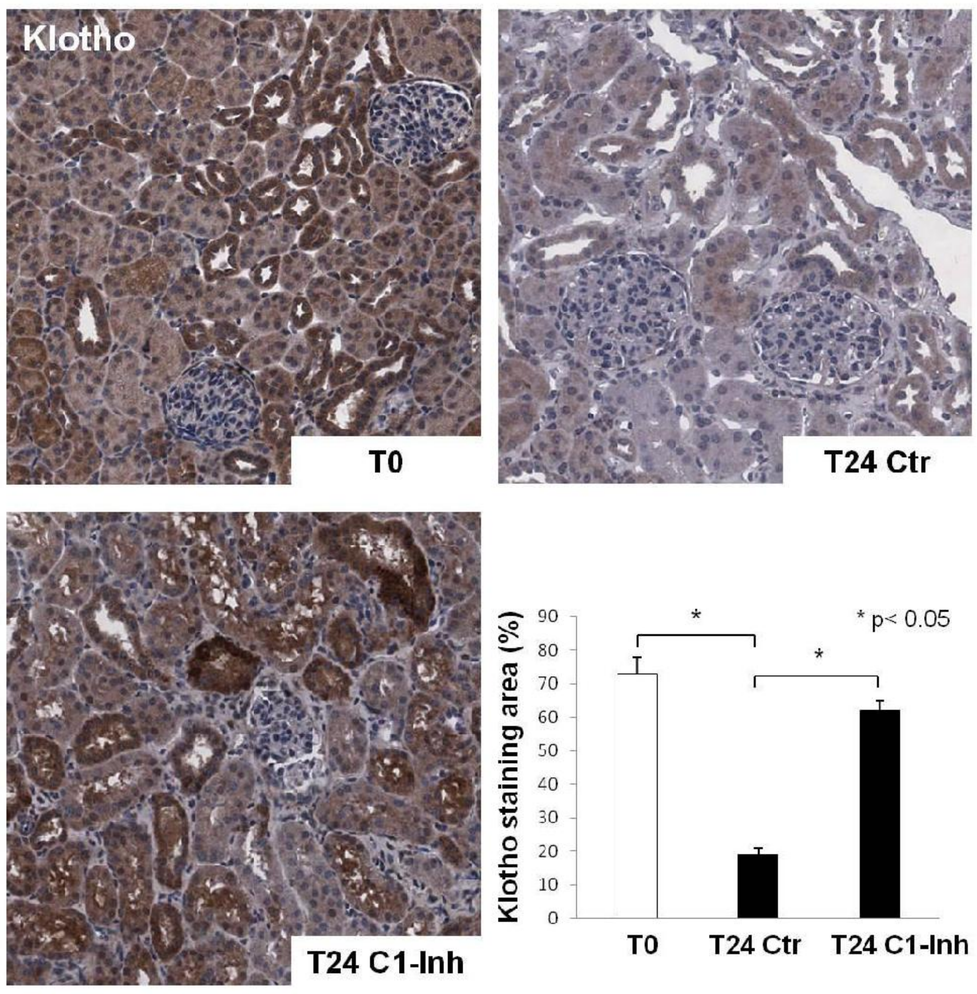

Fig1: KL was downregulated in a swine model of I/R injury after 24h of reperfusion compared to T0 C1-Inh treatment restored KL expression.

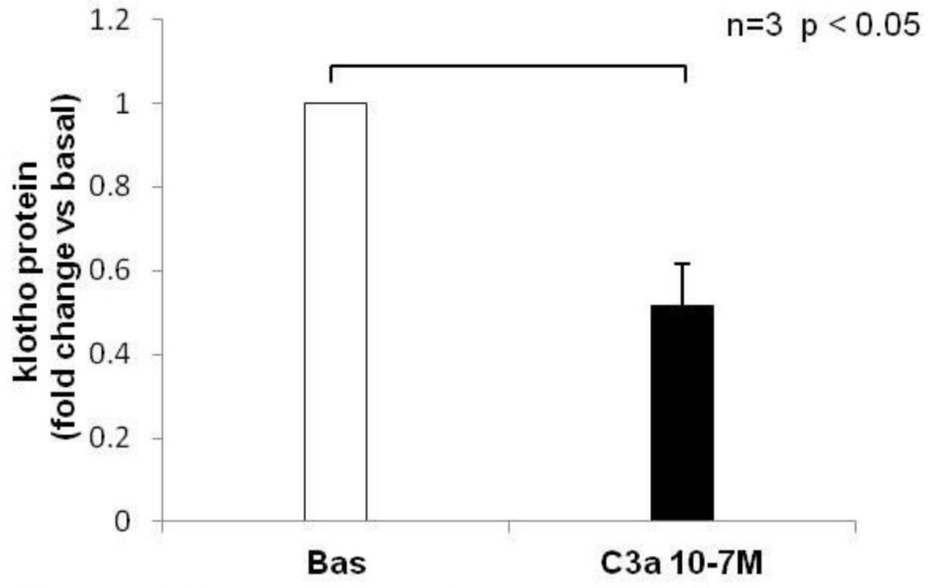

Fig2: In HK-2 cells, C3a considerably reduced KL protein expression compared to basal condition.

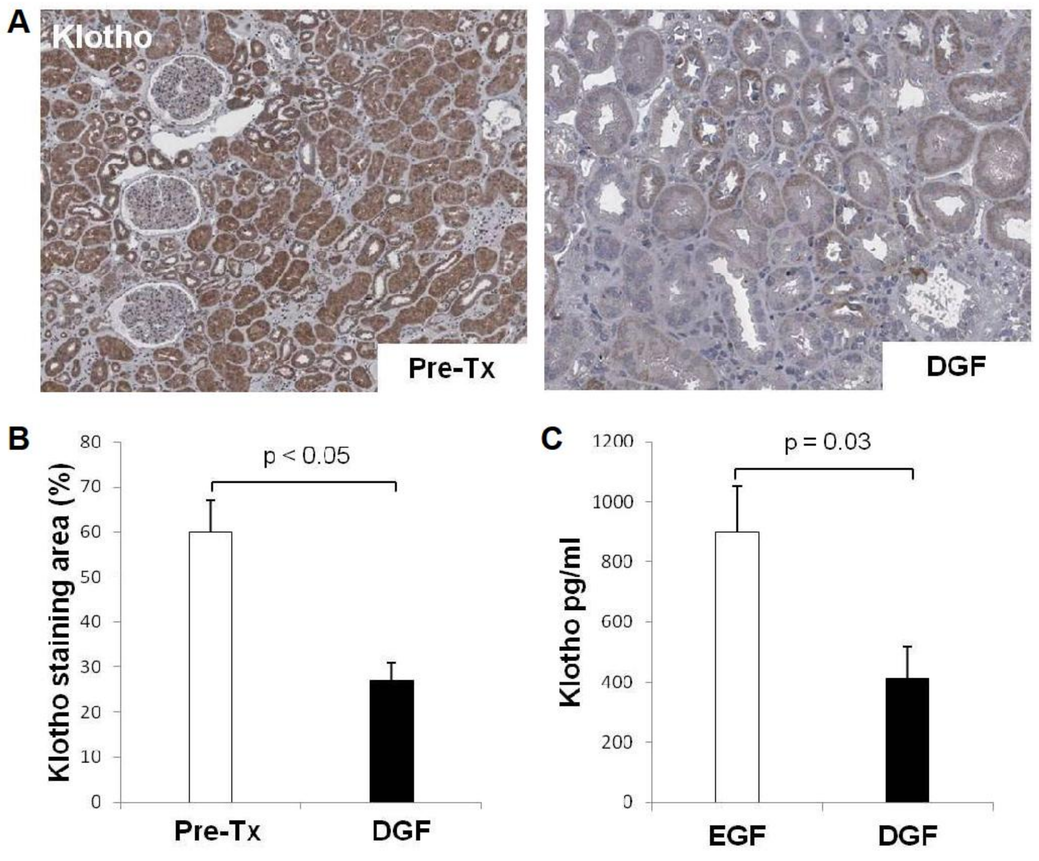

Fig3: Renal KL was downregulated in DGF biopsies compared to Pre-Tx condition (A-B). Soluble KL was reduced in serum of DGF patients respect to EGF patients after 2 years from transplantation (C).

References:

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G) Acute Renal Failure. G1) Experimental models.
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