The role of Calreticulin in renal fibrosis

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The Unilateral Ureteral Obstruction (UUO) model of kidney fibrosis

• in vivo model

• encompasses many aspects of other models of kidney fibrosis

• there are features that occur within 1 week

• mimics in a short time a situation that can take years in humans

• leaves one kidney intact

• there is evidence that animal models with UUO are reflective of the molecular changes in human situations
Proteomic analysis

Kypreou K. et al., *Proteomics*, 2008
Venn diagram of identified proteins

Sham / Ligated 2

28

1

2

9

Sham / Ligated 8

5

18

Ligated 2 / Ligated 8

2
Calreticulin is a multifunctional protein

- First isolated in 1974 as a high-affinity Ca\(^{2+}\)-binding protein of the ER

There is no correlation with fibrotic processes yet!
Confirmation of Calreticulin upregulation in fibrotic samples

Quantification of calreticulin protein expression

Quantification of calreticulin mRNA expression normalized to 18S rRNA

Kypreou K. et al., Proteomics, 2008
Calreticulin is upregulated in the tubular cells of the kidney

Sham operated  Ligated 2 days  Ligated 8 days
WHAT IS THE ROLE OF CALRETICULIN IN FIBROSIS?

- *Phenotypic observations in a tubular cell line after Calreticulin overexpression*
Calreticulin overexpression in HK-2 cells

- HK-2 cell lines, which stably express the mouse Calreticulin cDNA.
Calreticulin-overexpressing cells acquire an altered morphology

Control

Overexpression 2
Differentially expressed proteins in Calreticulin-overexpressing HK-2 cells

**E-cadherin to β-actin ratio**

**Vinculin to β-actin ratio**

**Vimentin to β-actin ratio**

- Control
- Overexpression 1
- Overexpression 2

**E-cadherin/β-actin expression**

**Vinculin/β-actin expression**

**Vimentin/β-actin expression**
Differentially expressed genes in Calreticulin-overexpressing cells

Expression of genes related to epithelial and mesenchymal phenotype

- E-cadherin
- Vimentin
- Acta2
- Snai1
- Snai2

Relative mRNA expression normalized to 18s rRNA

Control
- Overexpression 1
- Overexpression 2

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β-catenin expression/localization in the Calreticulin-overexpressing cells

pcDNA (control)  mCRT-16 (overexpression 1)  mCRT-17 (overexpression 2)

b-catenin
dapi
merge
Calreticulin overexpression induces the activity of the TCF-luc-reporter pTOPFlash
Calreticulin overexpressing cells have an increased secretory profile

Fibronectin to β-actin ratio

Control Overexpression 1 Overexpression 2

Fibronectin/β-actin

Fibronectin mRNA expression

Relative mRNA expression normalized to 18S rRNA

COL4A1 mRNA expression

Relative mRNA expression normalized to 18S rRNA
Collagen I expression levels are not increased by Calreticulin overexpression

Collagen I secretion in conditioned medium

**COL1A1 mRNA expression**

Ponceau S
Calreticulin overexpression enhances the migratory capacity of HK-2 cells
Calreticulin-overexpressing cells have lower proliferation capacity than the control cells.
Calreticulin-overexpressing cells exhibit increased cellular stress
Calreticulin overexpression enhances dramatically cell sensitivity to apoptosis

**TUNEL assay**

- % TUNEL counts/DAPI vs. control
- Control vs. Overexpression 2

**Activated caspase 3 expression**

- % activated caspase 3/DAPI
- Normal medium vs. H2O2 50μM

**TUNEL assay after treatment with H2O2**

- % TUNEL counts/DAPI vs. control
- Control vs. Overexpression 1 vs. Overexpression 2

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Conclusions

- Calreticulin overexpression in HK-2 cells:
  - increases fibronectin and collagen IV production
  - increases significantly the ER stress and the migration capacity
  - reduces proliferation rate while increases the cell sensitivity to apoptosis,
  - all of which represent characteristics of fibrosis.

- Calreticulin overexpression in HK-2 cells:
  - reduces E-cadherin expression
  - increases vimentin and vinculin expression
  - induces Snail1/2 expression and β-catenin activation
  - reduces aSMA expression while not affecting ColI production
  - thus resulting in a decline of the epithelial cell phenotype, without inducing complete EMT.

All the above points introduce Calreticulin as a novel potential central molecule in the fibrotic processes.
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