Characterization of a renal cell model by gene expression analysis

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Overview

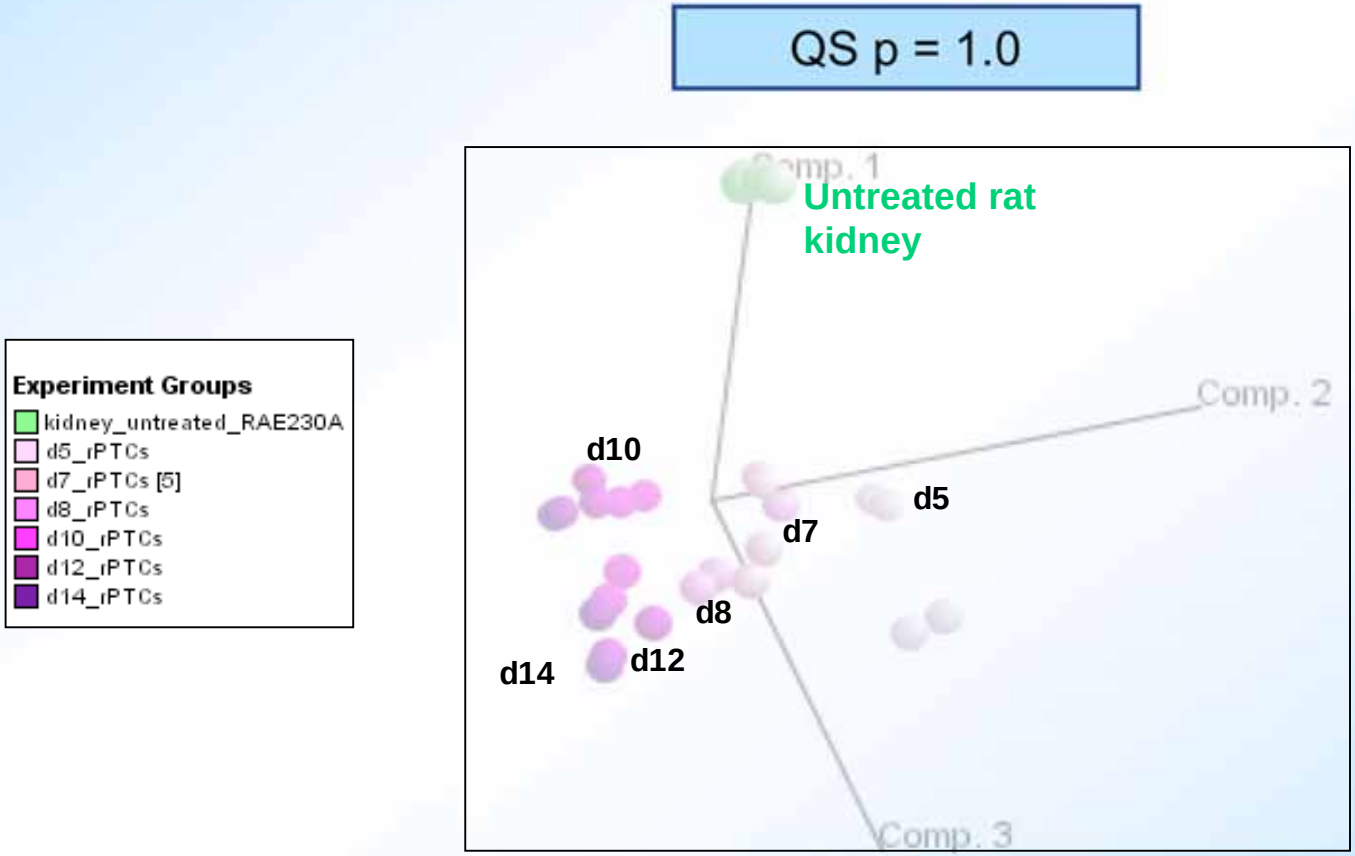
A) Basal gene expression of untreated primary rat proximal tubular cells (rPTCs) during culture in comparison to untreated rat kidney in vivo

B) Comparison of the genes deregulated after treatment with Ochratoxin A (OTA) in rPTCs, NRK-52E and rat kidney in vivo

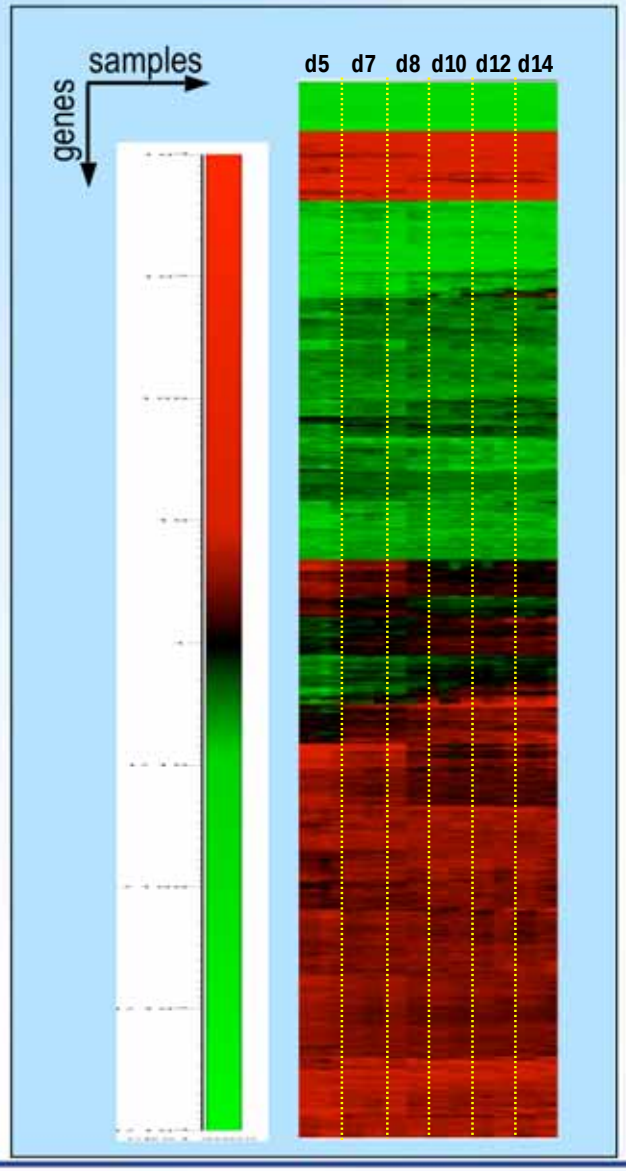
rPTC model vs in vivo - PCA Analysis - Overview

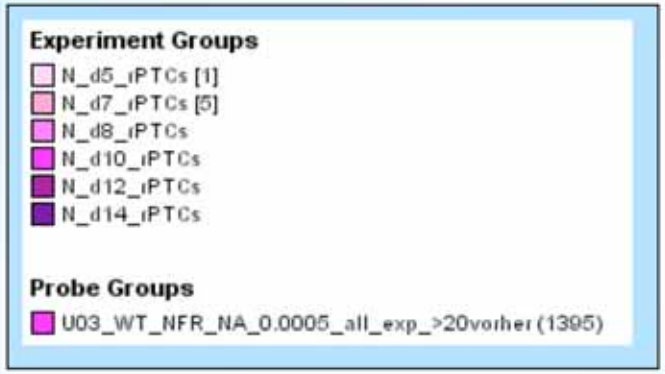
- **Comparison of cultured proximal tubular cells at different times after isolation with kidney *in vivo* in a Principal component analysis (PCA) based on the expression of of all genes (QS p = 1.0).**
- **4 biological replicates each**

QS p = 1.0



Rat Proximal Tubular Cells vs Kidney – Expression Profiles





1D hierarchical cluster analysis with relative data (normalized to kidney in vivo) of all cell culture time points. Main characteristics regarding the deregulation in cells vs kidney are:

- **Upregulation**
- **Downregulation**
- **Increase in culture**
- **Up day 5**

718 Genes upregulated in culture vs kidney

677 Genes downregulated in culture vs kidney

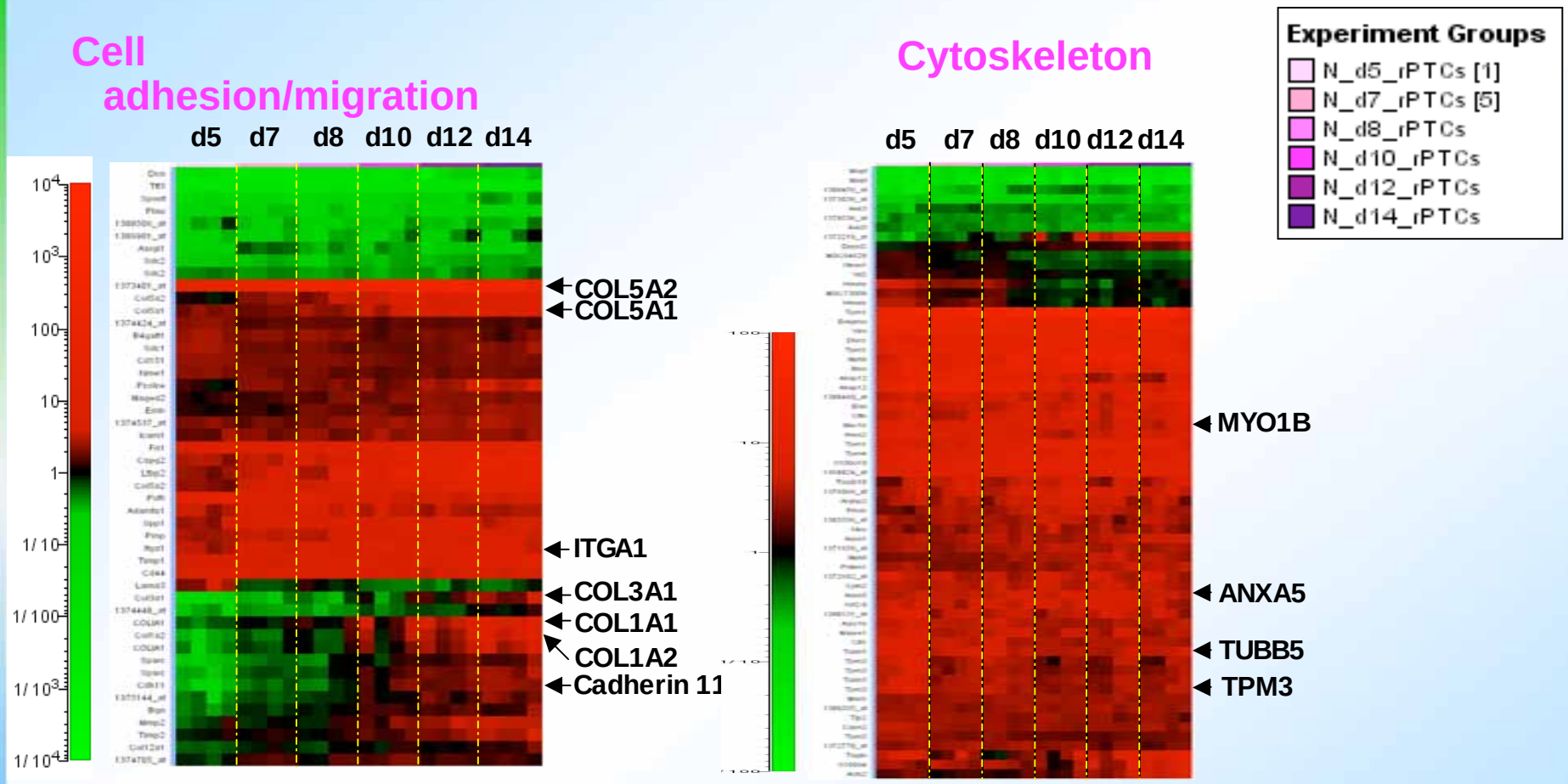
Gene Categories – Cell cycle/proliferation

Cell cycle

- Genes involved in G2/M Phase are significantly upregulated during day 5 to 8.
- Genes involved in G1/S Phase are upregulated after day 8
- Thus from day 5 – 14 the cells undergo one mitosis and potentially will undergo a 2nd mitosis after day 14

Proliferation

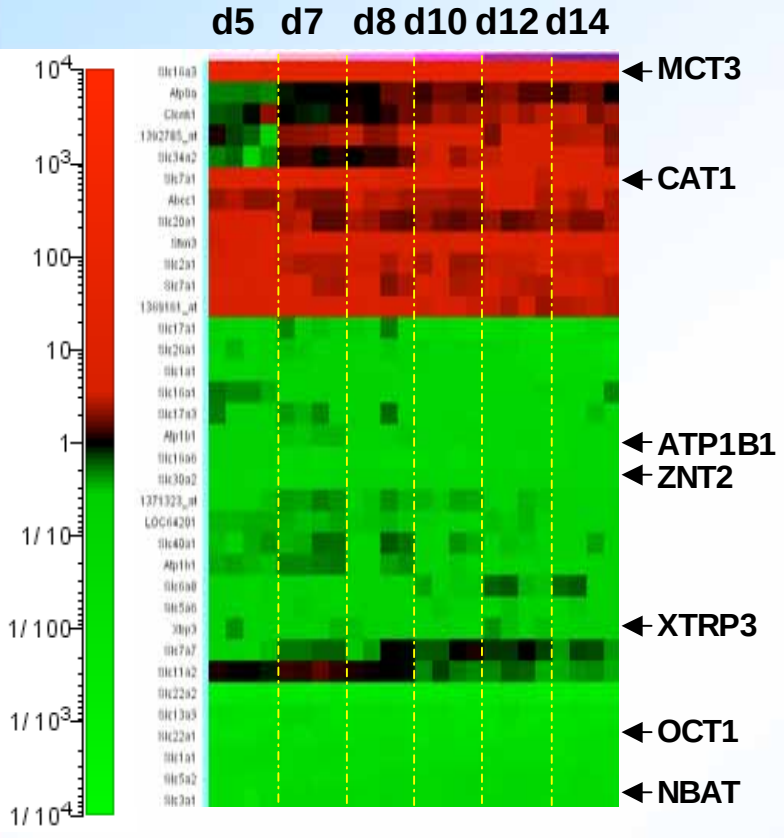
Gene Categories – Cell adhesion/migration | Cytoykeleton (2)



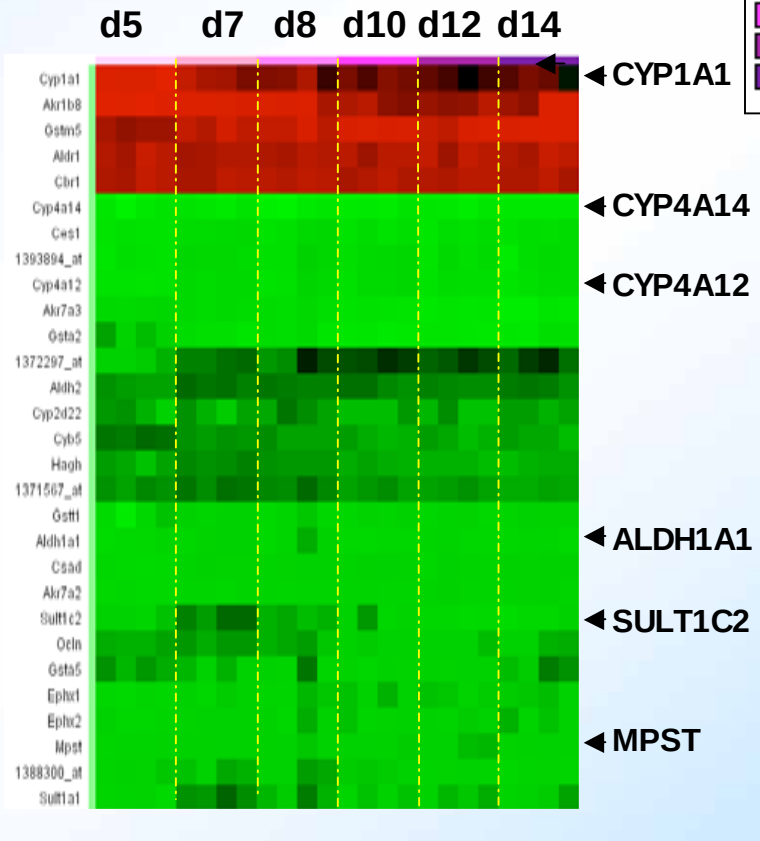
Adjustment to the in vitro environment, differentiation into a "tissue-culture"-type cell

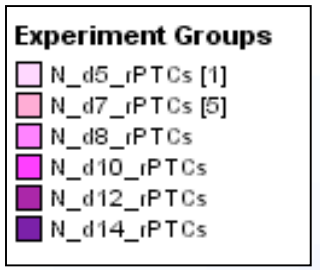
Gene Categories - Dedifferentiation

Dedifferentiation – membrane transporters



Dedifferentiation - Biotransformation





Dedifferentiation – loss of kidney-specific transporter activities

Dedifferentiation – loss of metabolic activities

Rat Kidney Proximal Tubular Cells vs Kidney – Gene categories

Main categories of genes downregulated in proximal tubular cells vs kidney

Amino Acid Metabolism
Biotransformation
Lipid metabolism
Carbohydrate metabolism
Energy metabolism

Dedifferentiation - loss of metabolic activity

Membrane transport
Ion homeostasis

Loss of kidney specific activities

Main categories of genes upregulated in proximal tubule cells vs kidney

Stress responses
Apoptosis

Stress responses to new environment

Metabolism
Protein metabolism
RNA metabolism
Intracellular transport
Cytoskeleton
Cell adhesion/migration

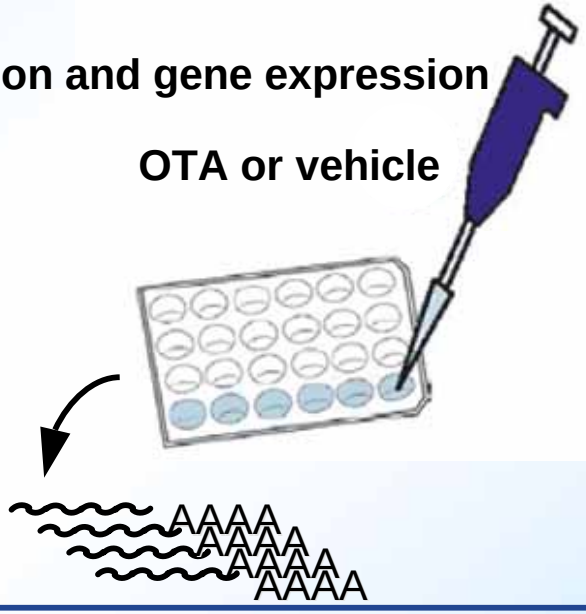
Synthesis and transport of new proteins
Adjustment to new environment
Differentiation into a tissue culture cell

Regulation of gene expression
Regulation of proliferation
DNA replication
Cell cycle/proliferation

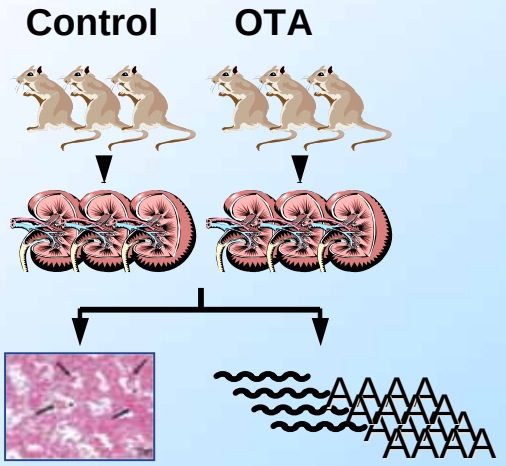
Proliferation in culture

Study Design - *in vitro* and *in vivo* treatment with OTA

- ***In vitro* dosing**:
  - Primary rat proximal tubular cells (rPTCs) from Wistar rats, 4 replicates per timepoint or
  - NRK-52E, rat kidney cell line, 3 replicates per time point
  - Time course: 1d, 3d
  - Compound
    - Ochratoxin A (OTA): 20 µM
  - Time-matched controls (vehicle: DMSO)
  - RNA isolation and gene expression analysis



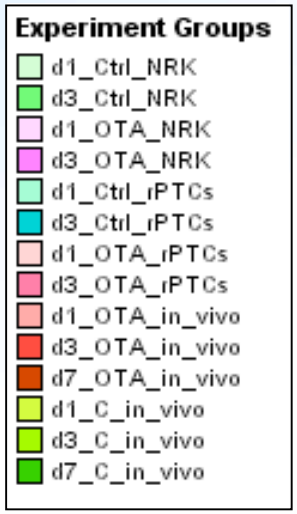
- ***In vivo* dosing**:
  - Male Wistar rats, 3 animals per dose-timepoint
  - Time course: 1d, 3d, 7d
  - Compound
    - Ochratoxin A (OTA): 3 mg/kg/day
  - Time-matched controls
  - Kidney:
    - Kidney cortex:
      - RNA isolation and gene expression analysis

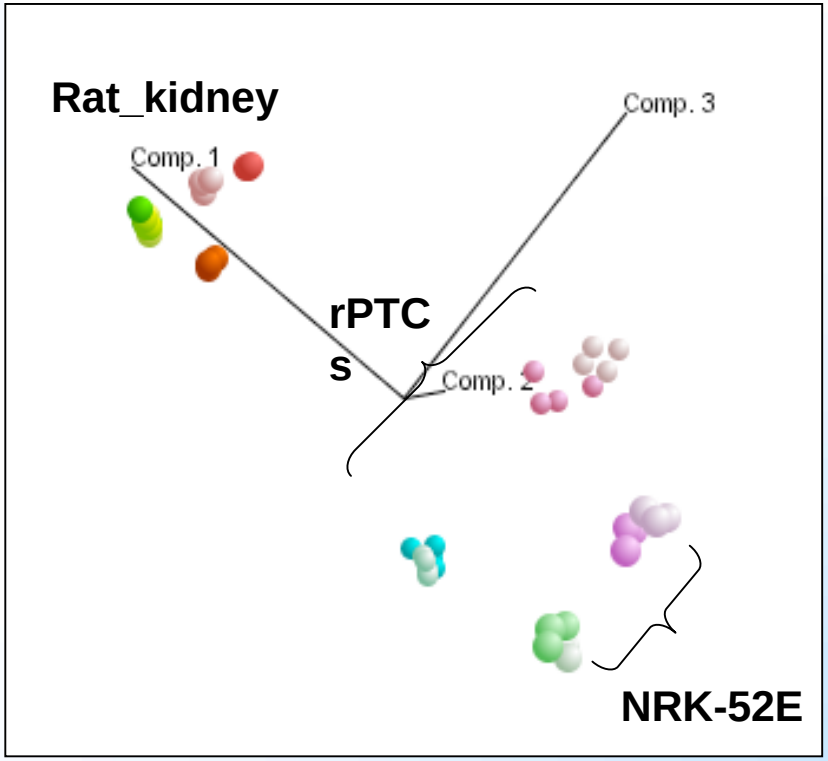


Comparison between *in vivo* and *in vitro* after treatment with OTA

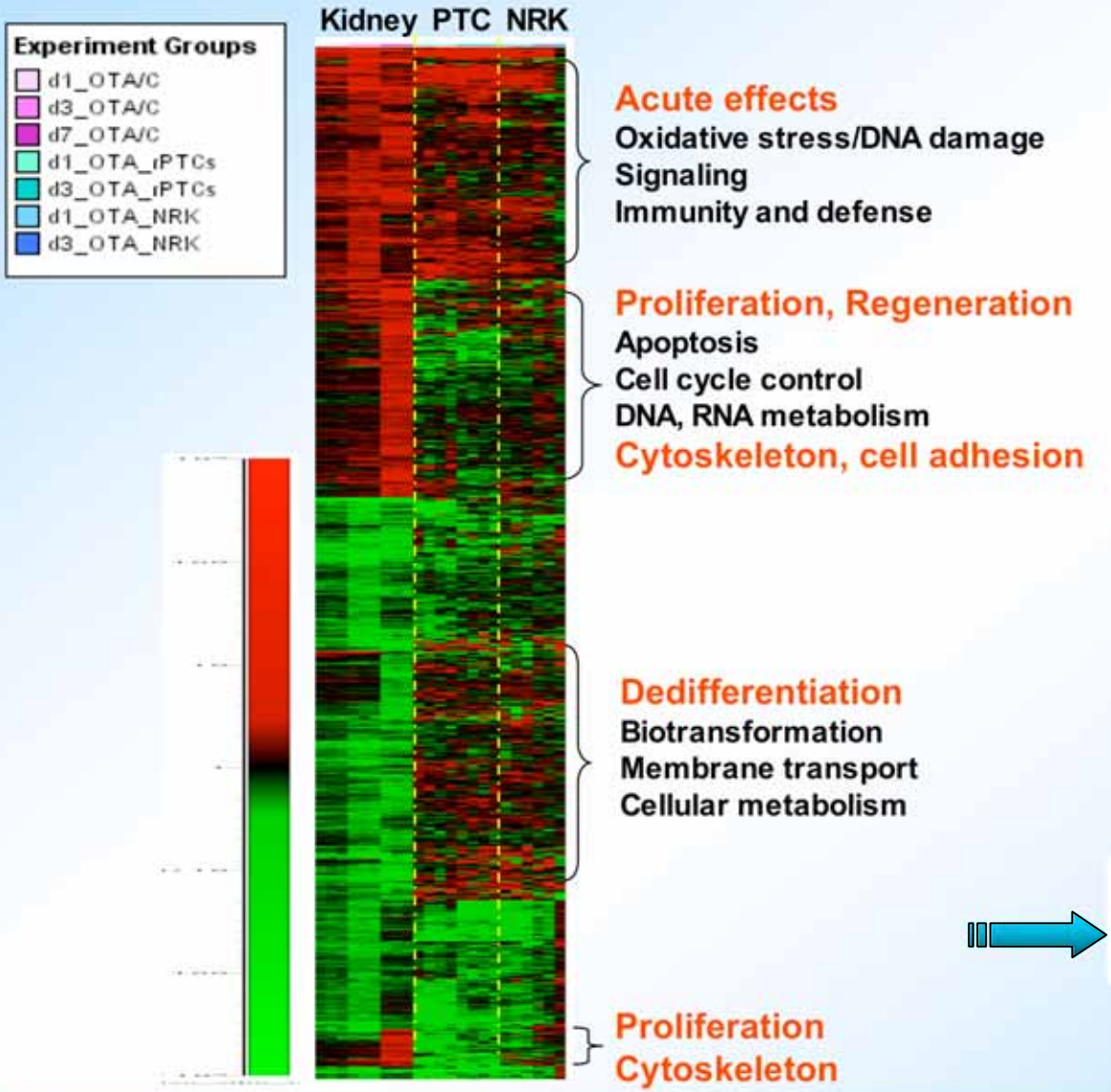
- **OTA-treatment induces gene expression changes in vivo and in culture**
- **Yet the differences between in vivo and in vitro models are > than between treated and control samples**

QS p = 1.0





Comparison between *in vivo* and *in vitro* after treatment with OTA



- **Genes:**
  Those significantly de-regulated by OTA in vivo
- **Kidney systems:**
  - **Rat kidney in vivo treated with OTA (3 mg/kg/day) for 1, 3, and 7 days**
  - **rPTCs treated with 20 µM OTA for 1 and 3 days.**
  - **NRK-52E treated with 20 µM OTA for 1 and 3 days**
- **All data are normalized to the time-matched and appropriate controls.**

Defined cell reactions are missing

Conclusion

- Cell systems have a defined phenotype which is different from in vivo
- Differences upon treatment:
  - Upregulated by OTA in vivo, not consistently deregulated in vitro

  → Regenerative responses appear to be lacking in vitro
    - Proliferation was already highly induced in vitro; no additional increase possible
  - Upregulated by OTA in vivo and in vitro

  →Acute stress responses seem to be functioning both in vivo and in vitro
  - Downregulated by OTA in vivo, some genes variably induced in vitro

  → Dedifferentiation upon OTA treatment in vivo
    - The basal level of these genes in vitro was already much lower than in vivo. Therefore downregulation was possible only in vivo
- Differences have to be taken into account when using cell systems

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PREDICTOMICS
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