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Integrated transcriptomic and proteomic evaluation of gentamicin nephrotoxicity in rats

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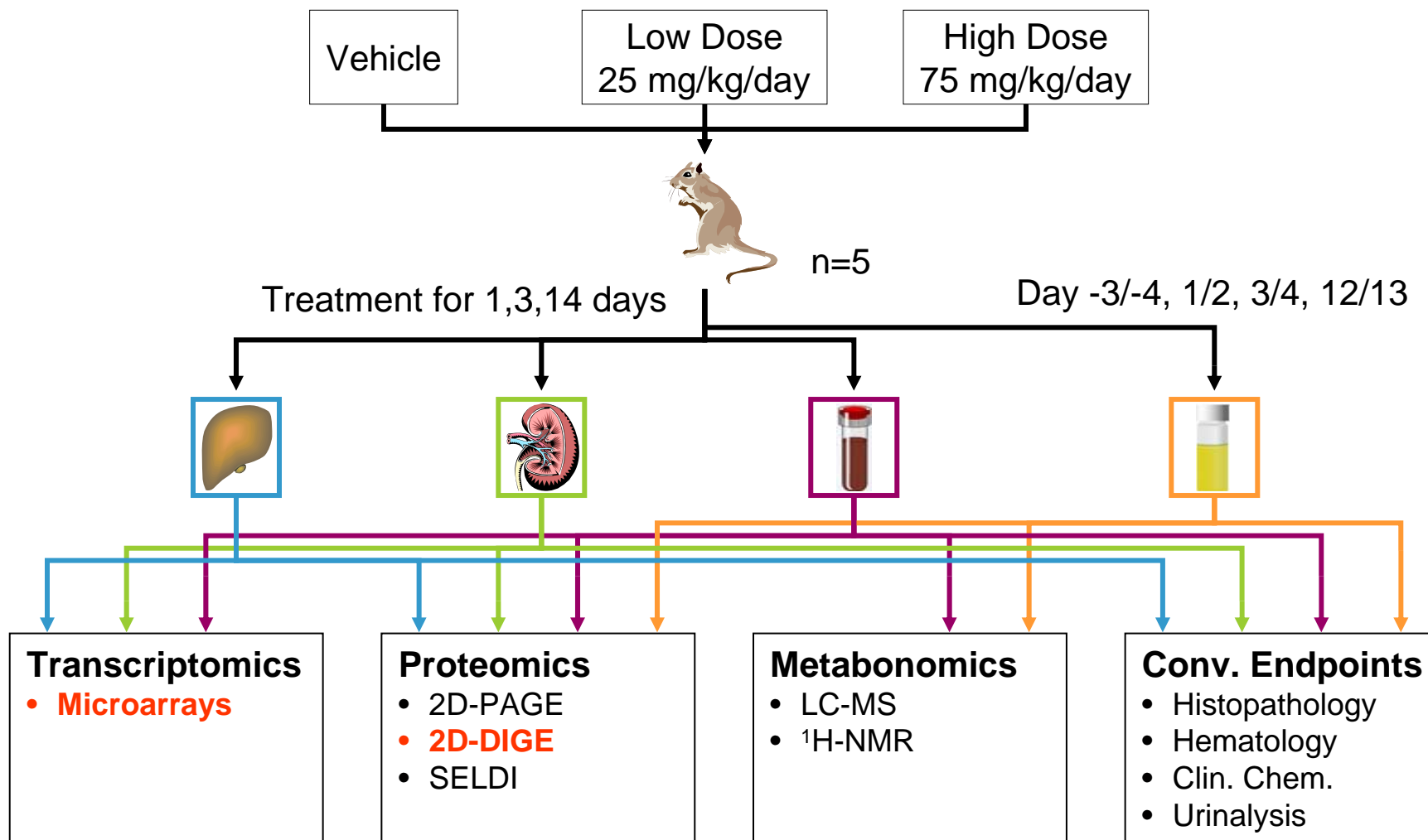
SOT 47th Annual Meeting, Seattle, March 18, 2008

Background information

- **Gentamicin is an aminoglycoside antibiotic which induces renal tubular necrosis in rats**
 - ▶ **Mechanisms of toxicity: inhibition of lysosomal phospholipases, mitochondrial dysfunction, oxidative stress, etc, ...**
- **Goal of InnoMed PredTox project :**
 - ▶ **Provide more informed decision making *earlier* in preclinical safety evaluation by combining results from ‘omics technologies together with conventional toxicology methods**
 - ▶ **Mechanistic investigation and biomarker identification**
- **Gentamicin was chosen as a reference nephrotoxicant in PredTox database**



Study design and investigations





Conventional toxicology results

- Decrease of mean body weight gain at 75 mg/kg at Day 15 resulting in lower mean body weight as compared to controls: - 8.6 %
- No biologically significant changes in clinical pathology parameters
- At Day 15 and 75 mg/kg/day, minimal to mild tubular **degeneration/necrosis** in kidney associated with mild to moderate **tubular regeneration** and minimal to moderate **mononuclear cell infiltrates** in all animals
- At Day 15 and 25 mg/kg/day, minimal tubular degeneration/necrosis associated with minimal tubular regeneration and minimal to mild mononuclear cell infiltrates in 2/5 animals

Key findings of kidney transcriptomic analysis

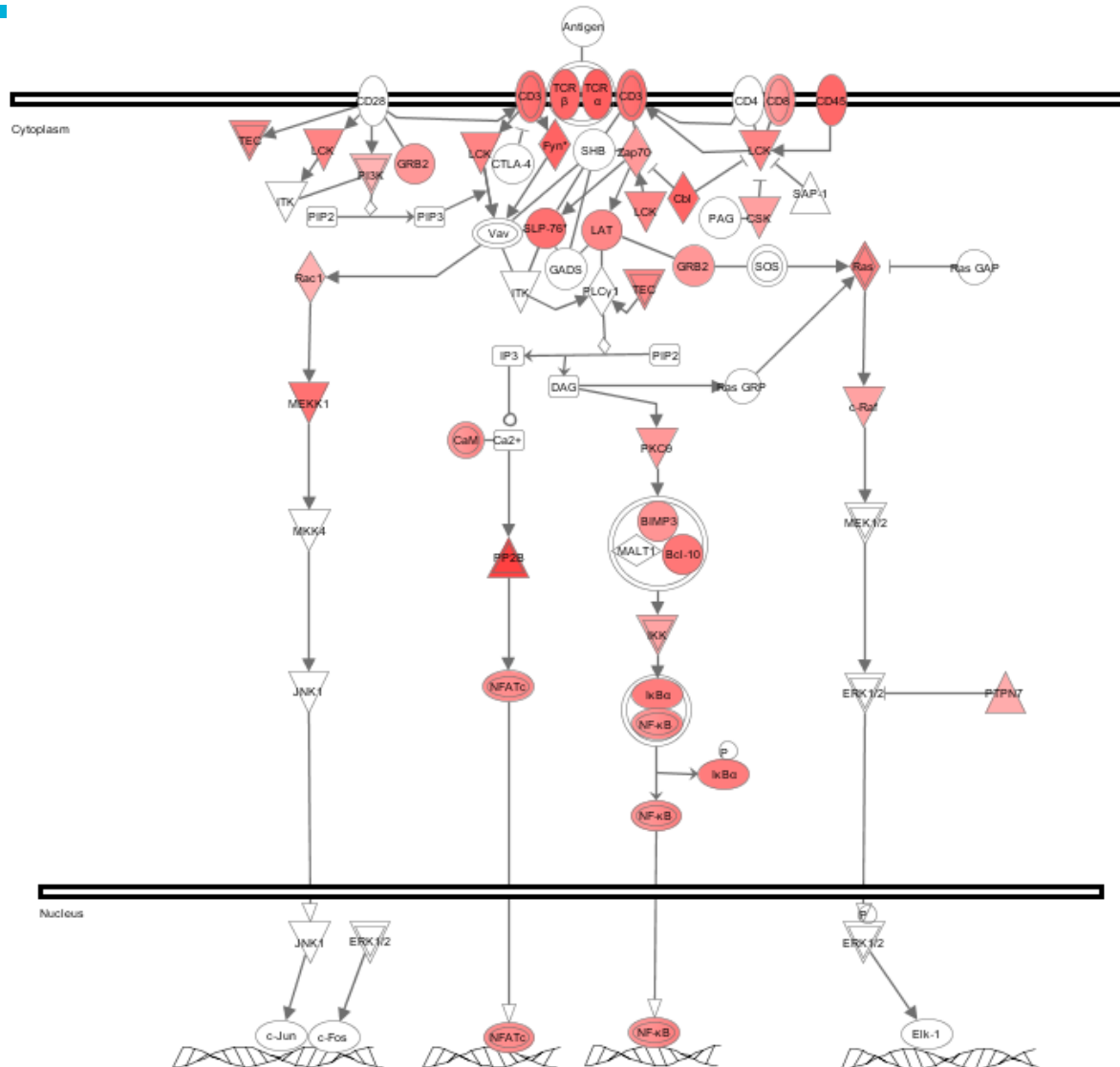
- Significant modulation of 463 genes (303 up and 160 down) only observed at the high dose of gentamicin after a 14-day treatment
- Regulated genes involved in several biological processes:
 - ▶ NF- κ B pathway through p38 MAPK cascade
 - ▶ Inflammation (e.g. chemokines, interleukin 7)
 - ▶ Apoptosis, protein catabolism
 - ▶ Anti-apoptosis signals, cell proliferation (e.g. Stat1, granulins and Kit ligand), protein synthesis
- The top up-regulated gene in kidney was the potential nephrotoxicity biomarker KIM-1
- No earlier effects of gentamicin
- Low dose of gentamicin did not modulate gene expression in the kidney

Key findings of blood transcriptomic analysis

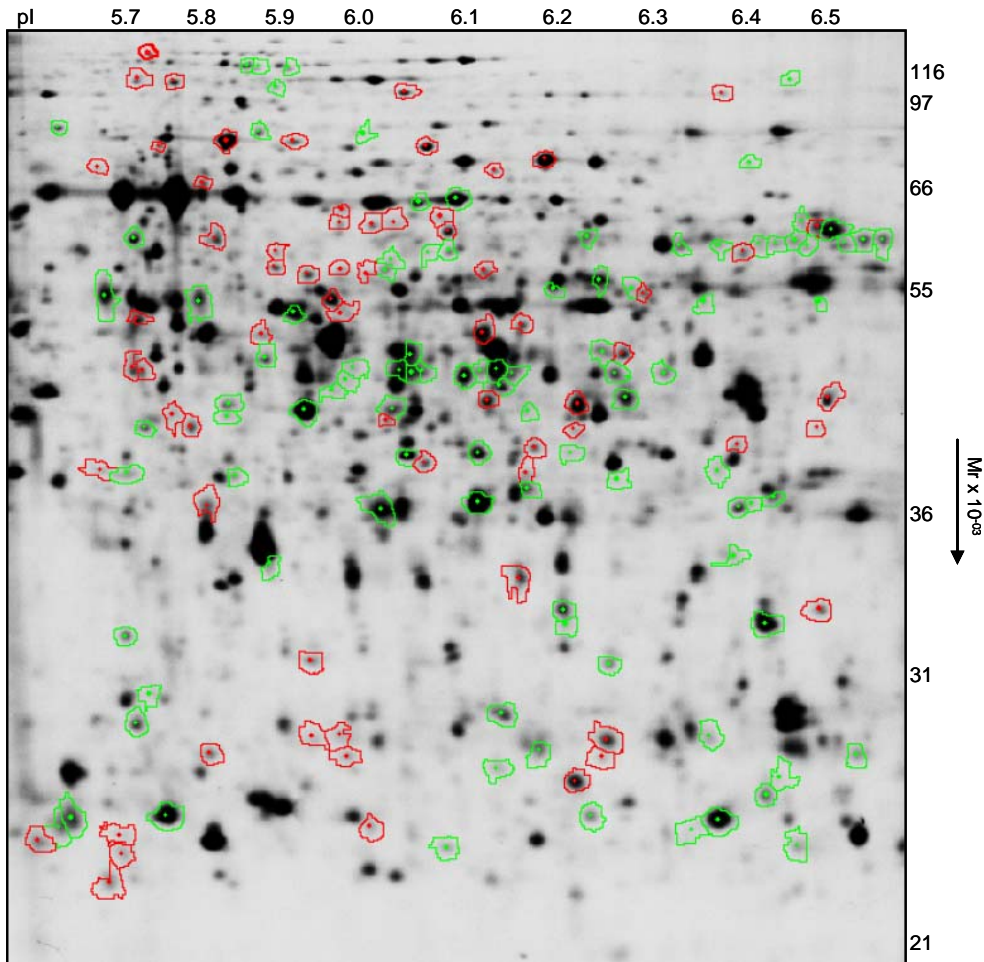
- **Significant modulation of 3246 genes (923 up and 2323 down) only observed at the high dose of gentamicin after a 14-day treatment**
- **Regulated genes involved in several biological processes:**
 - ▶ Inflammatory and immune response (antigen processing and presentation of peptide or polysaccharide antigen via MHC class II, T-cell activation)
 - ▶ Protein biosynthesis, DNA replication
 - ▶ Apoptosis, RNA and protein catabolism
- **No earlier effects of gentamicin**
- **Low dose of gentamicin did not modulate gene expression in the blood**



IPA analysis: T-Cell receptor signaling



2D-gel image analysis in kidney after a 14 day treatment with 75 mg/kg/day of gentamicin

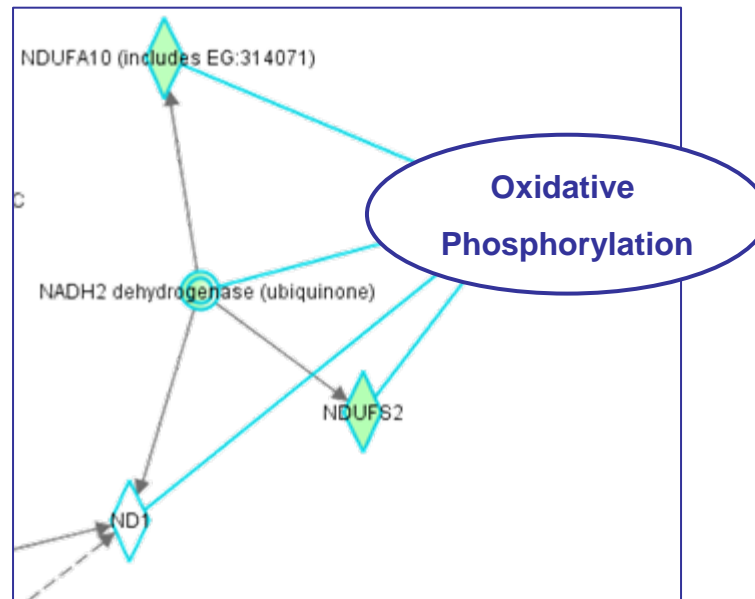


- **Detection of**
 - ▶ 96 down-regulated spots
 - ▶ 67 up-regulated spots
- **Identification of**
 - ▶ 56 different down-regulated proteins
 - ▶ 49 different up-regulated proteins



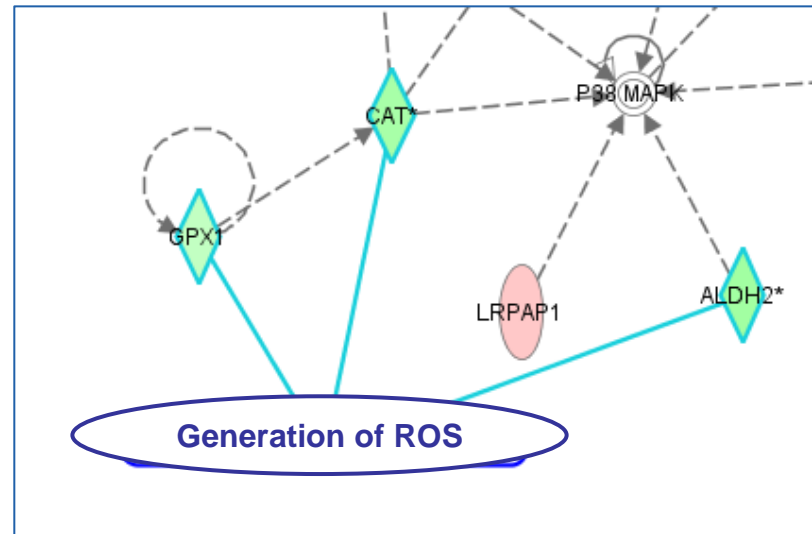
Mitochondrial dysfunction

- Around 25% of the modulated proteins were located in mitochondria
 - ▶ 11 up-regulated
 - ▶ 19 down-regulated
- IPA analysis: proteins involved in citrate cycle and oxidative phosphorylation → Possible impairment of cellular energy production



Induction of oxidative stress

- GO analysis: enrichment of response to oxidative stress
- Down regulation of antioxidant enzymes (**catalase, glutathione peroxidase 1, glutathione peroxidase 3, peroxiredoxin 1**)
- IPA analysis: down-regulation of 3 proteins which decrease the generation of reactive oxygen species (ROS)



► Increase of the level of ROS → oxidative stress



Other biological processes affected

Cell death

- ▶ **ENO1, NDRG1, G6PD, APRT**

Cellular assembly and organization

- ▶ **GSN, DPYSL2, PDCD6IP, ERM proteins (ezrin, radixin, moesin)**

Protein synthesis

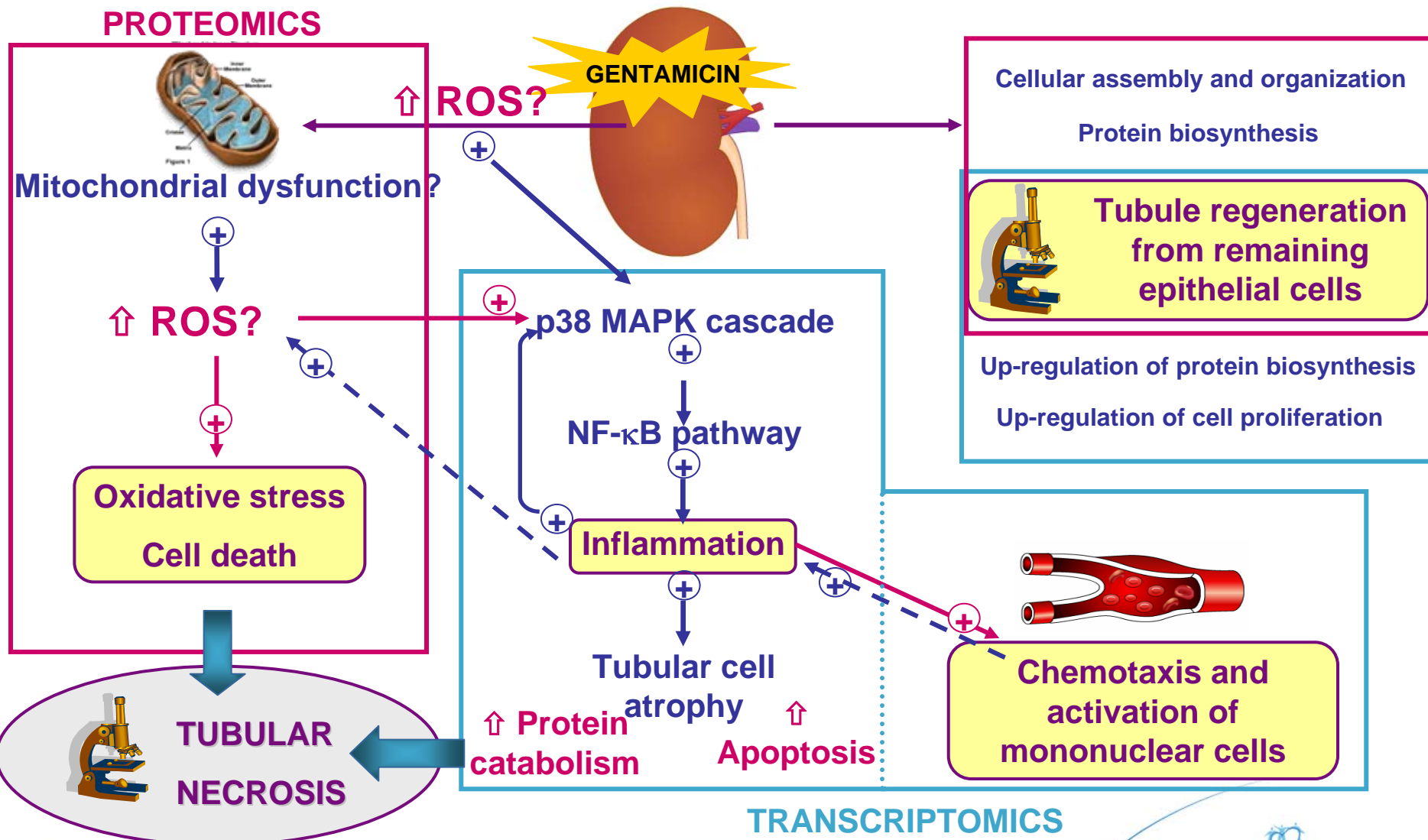
- ▶ **EIF3S3, EEF2, amino-acid tRNA synthetases, TUFM (mitochondrial)**

Protein modification and folding

- ▶ **CCT2, CCT3, CCT6A, MSRA**



Integration of transcriptomic and proteomic data: a putative mode of toxicity of gentamicin in kidney





Conclusions

- Modulation of kidney proteins involved in **possible mitochondrial dysfunction and oxidative stress** may be the consequence of toxicity
- Induction of **NF- κ B pathway** in kidney and **T-cell receptor signaling** in blood cells are in accordance with inflammation and could be responsible for the recruitment of mononuclear cells in kidney
- Modulation of genes and proteins involved in **cell proliferation, protein biosynthesis, cellular assembly and organization** are in agreement with the observed tubular regeneration in kidney
- Transcriptomic data in kidney/blood and proteomic data in kidney turned out to be complementary and gave insight into the putative mode of nephrotoxicity of gentamicin, in accordance with the observed histopathology



Next steps

- **Confirm modulated kidney proteins by Western-blot and examine earlier time-points**
- **Measure novel potential biomarkers of nephrotoxicity in urine (e.g. KIM-1, clusterin)**
- **Compare with metabonomic results in urine and plasma**
- **Perform cross-compound analysis with other nephrotoxicants within PredTox database**



Acknowledgements

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- ▶ Emmanuelle Com
- ▶ Eric Boitier
- ▶ Jean-Pierre Marchandeu
- ▶ Martine Courcol
- ▶ Jean-François Léonard
- ▶ Olivier Dorchies
- ▶ Bruno Genet
- ▶ Marc Duchesne

PredTox Consortium

- ▶ Susanne Schroeder
(Nycomed GmbH)
- ▶ Anrd Brandenburg
(Genedata)
- ▶ Maria Wendt (Genedata)
- ▶ Laura Suter-Dick (Roche)
- ▶ Friedlieb Pfannkuch (Roche)