Gene expression profiling in liver transplantation

Kim M. Olthoff, M.D.
Associate Professor of Surgery
Director, Liver Transplant Program
University of Pennsylvania
Gene expression profiling in liver transplantation

- Pubmed search
  - “Liver transplant and gene expression profiling”
  - 12 results: 2001-2006
Potential uses for gene expression profiling in liver transplantation

- **Pretransplant**
  - Predict clinical outcome in patients with HCC
  - Predict rate of fibrosis progression post OLT in HCV pts

- **Peri-transplant**
  - Assess function of deceased donor grafts in donors
  - Predict early graft dysfunction
  - Assess initiation of regeneration in LDs

- **Post-transplant**
  - Predict, determine rejection
  - Differentiate rejection from HCV, CMV
  - Assess HCV progression
Potential uses for gene expression profiling in liver transplantation

- **Pretransplant**
  - Predict clinical outcome in patients with HCC
      - Prospective multicenter trial now underway with A2ALL consortium
  - Predict rate of fibrosis progression post OLT
Potential uses for gene expression profiling in liver transplantation

- **Peri-transplant**
  - Assess function of deceased donor and living donor grafts
  - Predict early graft dysfunction
    - Olthoff/Salomon prelim data
      - Multicenter trial started (CTOT3)
  - Assess initiation of regeneration in LDs
    - Olthoff/Salomon prelim data
Potential uses for gene expression profiling in liver transplantation

- **Post-transplant**
  - Predict rejection/tolerance
  - Differentiate rejection from HCV, CMV
Potential uses for gene expression profiling in liver transplantation

- **Post-transplant (continued)**
  - Assess HCV progression of fibrosis
Limitations in liver array

- Heterogeneous cellular composition
- Paracrine actions between different cell types
- Complexity of hepatic transcriptome
- Expanding clinical variables
- Statistical analysis and difficulties in validating results in terms of real biologic significance
Human liver regeneration and transplantation

- Why is regeneration required after liver transplantation?
  - Replacement of injured cells
    - Cold ischemia/reperfusion injury
    - Immune injury
  - Growth of insufficient liver mass
    - Small for size whole grafts
    - Cadaveric split grafts and living donor hemi-grafts
Differences between whole and partial liver grafts

<table>
<thead>
<tr>
<th></th>
<th>LD: partial graft</th>
<th>DD: whole graft</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic mass</td>
<td>40-60% of SLV</td>
<td>Sufficient</td>
</tr>
<tr>
<td>IR injury</td>
<td>Minimal</td>
<td>Moderate to extensive</td>
</tr>
<tr>
<td>Expected</td>
<td>Regeneration</td>
<td>Inflammation, repair</td>
</tr>
</tbody>
</table>
Liver function and injury: 
Balance of metabolism and recovery

Metabolism:
- Synthesis
- Storage and redistribution
- Protein production

Recovery from:
- Necrosis
- Apoptosis
- Inflammation

Need for:
- Regeneration
- Remodeling

Energy

Detoxification
Homeostasis
Patterns of gene expression in human living donor (LD) liver transplantation compared to deceased donors (DD)

Pilot Study at UPENN, Scripps collaboration

- **Aim:**
  - to determine differences in gene expression profiles between LD and DD liver grafts

- **Patients:**
  - 21 patients consented
  - matched for indication for transplant and donor characteristics:
    - 8 LD patients
    - 13 DD patients
Methods:
Timing of liver biopsies

**DDLT**

- Donor procedure
- Bench surgery
- Reperfusion
- Cold ischemia
- Warm ischemia

**LDLT**

- Donor procedure
- Bench surgery
- Reperfusion
- Splitting of the liver
- Cold ischemia
- Warm ischemia
Microarray analysis:
work-up of RNA following Affymetrix protocol

- Extraction of RNA using TRIzol / RNeasy
- Reverse transcription into cDNA
- In vitro transcription into biotin labeled cRNA
- Affymetrix U133 2.0 plus gene chip
- Confocal laser scanning
- RMA express for normalization
- BRB Tools comparison: significance P<0.001
- EASE / DAVID data mining
- Ingenuity analysis
LD vs. DD:
Summary of class comparisons and differential gene expression

<table>
<thead>
<tr>
<th>Series of Class Comparisons in Liver Graft Biopsies</th>
<th>No. of Genes Up-regulated</th>
<th>No. of Genes Down-regulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class comparison (p&lt;0.005)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD-COLD vs. LD-PRE</td>
<td>110</td>
<td>99</td>
</tr>
<tr>
<td>DD-COLD vs. DD-PRE</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>LD-POST vs. LD-COLD</td>
<td>217</td>
<td>115</td>
</tr>
<tr>
<td>DD-POST vs. DD-COLD</td>
<td>444</td>
<td>117</td>
</tr>
<tr>
<td>LD-POST vs. LD-PRE</td>
<td>742</td>
<td>906</td>
</tr>
<tr>
<td>DD-POST vs. DD-PRE</td>
<td>505</td>
<td>236</td>
</tr>
<tr>
<td>DD-PRE vs. LD-PRE</td>
<td>439</td>
<td>570</td>
</tr>
<tr>
<td>DD-POST vs. LD-POST</td>
<td>868</td>
<td>835</td>
</tr>
</tbody>
</table>
Results 1:
Differentially expressed genes in Deceased-donor grafts

- DD COLD vs PRE: up 5 genes, down 5 genes (10)
- DD POST vs PRE: up 505 genes, down 236 genes (741)
Results 1: Deceased donors
Up-regulated in DD POST vs. PRE

<table>
<thead>
<tr>
<th>Biological process</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transcription regulator activity</td>
<td>5.75E-12</td>
</tr>
<tr>
<td>Transcription factor activity</td>
<td>3.92E-10</td>
</tr>
<tr>
<td>Cell proliferation</td>
<td>4.56E-10</td>
</tr>
<tr>
<td>Response to stress</td>
<td>1.57E-08</td>
</tr>
<tr>
<td>Regulation of cell cycle</td>
<td>2.79E-08</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>3.35E-08</td>
</tr>
<tr>
<td>Inflammatory response</td>
<td>7.66E-08</td>
</tr>
<tr>
<td>Immune response</td>
<td>2.44E-07</td>
</tr>
</tbody>
</table>
Results 1: Deceased donors
Down-regulated in DD POST vs. PRE

<table>
<thead>
<tr>
<th>Biological process</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell organization</td>
<td>1.68E-03</td>
</tr>
<tr>
<td>Maintenance of chromatin architecture</td>
<td>9.24E-03</td>
</tr>
<tr>
<td>DNA packaging</td>
<td>1.34E-02</td>
</tr>
<tr>
<td>Nucleobase, nucleoside, nucleotide and nucleic acid metabolism</td>
<td>2.55E-02</td>
</tr>
<tr>
<td>DNA metabolism</td>
<td>3.90E-02</td>
</tr>
</tbody>
</table>
Results 2: Differentially expressed genes in living-donor grafts

- LD COLD vs PRE: up 110, down 99 (209)
- LD POST vs PRE: up 742, down 906 (1648)
## Results 2: Living donors

**Up-regulated in LD POST vs. PRE**

<table>
<thead>
<tr>
<th>Biological process</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA metabolism and processing</td>
<td>2.05E-17</td>
</tr>
<tr>
<td>Ribosome biogenesis and assembly</td>
<td>1.28E-07</td>
</tr>
<tr>
<td>purine nucleotide binding</td>
<td>6.37E-07</td>
</tr>
<tr>
<td>mRNA metabolism and processing</td>
<td>5.56E-06</td>
</tr>
<tr>
<td>Translation</td>
<td>9.6E-06</td>
</tr>
<tr>
<td>Nucleobase, nucleoside, nucleotide and nucleic acid metabolism</td>
<td>2.29E-04</td>
</tr>
<tr>
<td>Regulation of cell cycle</td>
<td>4.51E-04</td>
</tr>
<tr>
<td>Protein metabolism</td>
<td>5.68E-04</td>
</tr>
</tbody>
</table>
### Results 2: Living donors

**Down-regulated in LD POST vs. PRE**

<table>
<thead>
<tr>
<th>Biological process</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid metabolism</td>
<td>3.44-12</td>
</tr>
<tr>
<td>Alcohol metabolism</td>
<td>1.79-10</td>
</tr>
<tr>
<td>Amino acid and derivative metabolism</td>
<td>5.67-10</td>
</tr>
<tr>
<td>Fatty acid metabolism</td>
<td>2.37-07</td>
</tr>
<tr>
<td>Carbohydrate metabolism</td>
<td>1.25-04</td>
</tr>
<tr>
<td>Energy pathways</td>
<td>5.43-04</td>
</tr>
<tr>
<td>Electron transport</td>
<td>6.49-04</td>
</tr>
<tr>
<td>Steroid metabolism</td>
<td>6.23-03</td>
</tr>
</tbody>
</table>
DD vs. LD Comparison:
Differentially expressed genes after reperfusion

Living donors: 1435 genes
Deceased donors: 527 genes
Common probe sets: 214 genes
Results 3: Biological function analysis

**Up-regulated in LD compared to DD**

<table>
<thead>
<tr>
<th>Biological process</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA metabolism and processing</td>
<td>1.05E-04</td>
</tr>
<tr>
<td>Nucleobase, nucleoside, nucleotide and nucleic acid metabolism</td>
<td>3.07E-03</td>
</tr>
<tr>
<td>Antigen processing</td>
<td>1.24E-02</td>
</tr>
<tr>
<td>Transcription from Pol II promoter</td>
<td>1.67E-02</td>
</tr>
<tr>
<td>rRNA metabolism</td>
<td>3.38E-02</td>
</tr>
<tr>
<td>Phosphorylation</td>
<td>4.46E-02</td>
</tr>
</tbody>
</table>
Results 3: Biological function analysis

**Down-regulated in LD compared to DD**

<table>
<thead>
<tr>
<th>Biological process</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid metabolism</td>
<td>4.52E-03</td>
</tr>
<tr>
<td>Alcohol metabolism</td>
<td>1.20E-02</td>
</tr>
<tr>
<td>Biosynthesis</td>
<td>1.83E-02</td>
</tr>
<tr>
<td>Carbohydrate metabolism</td>
<td>1.86E-02</td>
</tr>
<tr>
<td>Energy pathways</td>
<td>2.00E-02</td>
</tr>
<tr>
<td>NAD(P)H dehydrogenase (quinone) activity</td>
<td>2.58E-02</td>
</tr>
</tbody>
</table>
Ingenuity analysis of genes differentially expressed in LD POST grafts compared to LD PRE grafts for the IL-6/NF-kB/GPCR network (Network 4).
Ingenuity analysis of genes differentially expressed in LD POST grafts compared to LD PRE grafts for the Metabolism network (Network 6).
Ingenuity analysis of genes differentially expressed in DD POST grafts compared to DD PRE grafts for the IGFBP/SOCS/IL-6 network (Network 2).
DD vs. LD Post

- Decreased expression of IL-6, NFκB pathway in DD

IL-6 and NFκB pathway
Results:
Confirmation microarray with qPCR

90% agreement in over 20 randomly picked genes
### Class comparisons

#### Primary graft dysfunction

<table>
<thead>
<tr>
<th></th>
<th>PS</th>
<th>genes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PNF Cold vs DD Cold</strong></td>
<td>up</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>down</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td><strong>PNF POST vs DD POST</strong></td>
<td>up</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>down</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
</tr>
</tbody>
</table>
Summary class comparisons
Different patterns of gene induction

● LD:
  ● During resection induction of DNA, RNA, protein synthesis
  ● After reperfusion up-regulation of RNA metabolism and incorporation of nucleotides, induction of pro-inflammatory and cell cycle genes, and alterations in glucose and lipid metabolism

● DD:
  ● In donor up-regulated oxidative phosphorylation
  ● Only small changes during cold ischemic preservation
  ● Up-regulation of inflammation and response to stress, cell cycle regulation and apoptosis
  ● No major down-regulation of metabolic genes
Summary

- There are significantly different gene expression profiles in LD and DD liver grafts. Differentially expressed genes and biologic pathways correlate with previously described molecular pathways in animals.

- Further defining these differences in transcriptional activation of functional gene networks is a first step in understanding the effects and interplay of inflammation and regeneration.

- Gene expression profiling is a powerful tool to identify and further dissect numerous clinical issues in liver transplantation, which may lead to development of therapeutic interventions.
Acknowledgements

- The PENN liver transplant lab and fellows 2003-2005
  - Jeroen deJonge
  - Fotini Debonera
  - Jinfu Xie
  - Guodong Wang
  - Dong Xin
  - Paige Porrett
  - Adam Frank
  - Heidi Yeh
  - Peter Abt
  - Kerem Bortecen

- TSRI Collaborators
  - Dan Salomon
  - Sunil Kurian

- CHOP Collaborators
  - Wayne Hancock