Microarray Analysis and Liver Diseases

Snorri S. Thorgeirsson M.D., Ph.D.

Laboratory of Experimental Carcinogenesis Center for Cancer Research, NCI, NIH
Application of Microarrays to Cancer Research

- Identifying genes and pathways in particular tumors or subset of tumors during tumorigenesis
- Diagnostic classification of tumors (class comparison)
- Discovering subsets of tumors (class discovery)
- Prediction of clinical outcome of tumors (class prediction)
- Discovering therapeutic targets
- Customized therapy for individual
HCC in USA

- HCC has doubled in the US over the past two decades
- HCC will continue to rise in the US
  - Affecting men and women and all ethnic groups
  - White men between 45 and 65 affected the most
- HCV-related HCC explains a large proportion of the rise
- Diabetes/obesity potentially important risk factors
**Pathogenesis of Human HCC**

- Differentiate between RN, LGDN, HGDN and early HCC (<2-3 cm) by gene expression profiles
- Predict early malignant conversion of HGDN
- Identify key oncogenic pathways driving malignant conversion
- Identify prognostic markers in both preneoplastic lesions and serum

**Goals**

- Diagnostic classification of tumors
- Discovering subsets of tumors
- Prediction of clinical outcome of tumors
- Identify therapeutic targets
- Identifying critical genes and pathways in subset of tumors
- Individualize therapy
Integrative Functional Genomics

Gene expression (human)  Gene expression (Animal models)

Personalized Medicine

Promoter analysis  CGH
Integrative Functional Genomics of Human HCC

Integration of gene expression data
Supervised and unsupervised analysis of data

Clinicopathological data
- histology
- tumor grade
- recurrence
- survival
- liver function
- metastasis
- vascular invasion

Identify best-fit mouse models for each subclass of human HCC
Identify conserved gene expression patterns in mouse and human HCC
Identify potential therapeutic targets in human HCC

Test hypothesis
Build hypothesis

Stratify HCC patients (Molecular classification)

Treatment without molecular classification
Treatment X
Treatment Y

Validation of targets In Vitro
Cell Lines

Validation of targets In Vivo
Mouse Models

Clinical Trials

Independent Gene Expression Signatures
- STEM CELLS
- LIVER REGENERATION
- SIGNALING PATHWAYS
- ONCOGENES
Topics

• Stem Cells and HCC
• Preneoplastic Lesions in HCC
  - Human HCC
  - Mouse HCC
Stem Cells and Liver Cancer

Ju-Seog Lee

Nature Medicine, 12(4): 410-6, 2006
Normal Stem Cell

Embryonic precursor

Normal fetal stem cell

Normal adult stem cell

Self-renewal

Differentiation

Mature Cells
(limited proliferative potential)

Cancer Stem Cell

Normal stem cell

Premalignant stem cell

Restricted Differentiation

“Benign” Cancer
(limited proliferative potential)

Mutagenesis

Restricted progenitor

Self-renewal

Malignant cancer stem cell
• Cancer cells evolve from normal cells following accumulation of genetic and epigenetic alterations.

• Gene expression patterns in cancer cells fatefuly reflects these alterations. However, significant fraction of gene expression program in the cancer cell is characteristic for the original normal cellular lineages (Nature 2000, 403:503-511).

• Neither physiological adaptation of cancer cells in vivo nor experimental adaptation in vitro is sufficient to completely overwrite the gene expression programs established during development (Nat. Genet. 2000, 24:227-235)
Hypothesis

If part of the gene expression program of non-transformed cellular lineages is retained in cancer cells, it may be possible to identify the cellular origin of cancer by cross-comparing cell lineage-specific gene expression signatures from animal models (or human tissues) with those of human tumors.

To test the hypothesis.....

Used gene expression patterns (“signatures”) of liver progenitor cells and hepatocytes from rat and mouse models to identify the cellular origin of human HCC.
Genes Shared Between Human and Mouse HCC Gene List and Rat Hepatoblast Gene List

80 genes
Cluster Analysis of Shared Genes (80 genes) in Human and Mouse HCC and Rat Hepatoblast

Rat: 9 samples
Human: 61 tissues
Mouse: 39 tissues
Survival Analysis of Patients With or Without Hepatoblast Gene Expression Signature

Kaplan-Meier plot for HCC (China), n=61

p = 1.0 \times 10^{-3}
Survival Analysis of Patients With or Without Hepatoblast Gene Expression Signature

Kaplan-Meier plot for HCC (China), n=61

\[ p = 7.03 \times 10^{-4} \]

Cluster B & HB- patients
N = 30 (16 Death)

Cluster A & HB- patients
N = 17 (14 Death)

Cluster A & HB+ patients
N = 14 (13 Death)
Class Prediction of Human HCC with Hepatoblast Gene Expression Signature

HCC Patients (n = 139)

- Training (n = 61)
  - Chinese HCC
  - Integration of data and cluster analysis
    - Differentially Expressed Genes (HB vs. HC)
  - Training Prediction Models (6 Classifiers)
    - HCA (Hierarchical Clustering Analysis)
    - LDA (Linear Discriminant Analysis)
    - SVM (Support Vector Machines)
    - NC (Nearest Centroid)
    - 3NN (3-Nearest Neighbor)
    - CCP (Compound Covariate Predictor)

- Test (n = 78)
  - Caucasian HCC

Survival Analysis

Kaplan-Meier plot for Chinese HCC (n = 51)
- HB+ patients N = 47 (30 Death)
- HB+ patients N = 14 (13 Death)

p = 1.9 x 10^{-7}

Kaplan-Meier plot for Caucasian HCC (n = 52)
- HB+ patients N = 44 (23 Death)
- HB+ patients N = 8 (6 Death)

p = 4.7 x 10^{-8}

HCC (China)

HCC (Belgium, Mayo)
Three Subclasses of HCC Patients

Kaplan-Meier plot for All HCC (n = 113)

\[ p = 3.74 \times 10^{-6} \]

Cluster B & HB- patients
N = 56 (29 Death)

Cluster A & HB- patients
N = 35 (24 Death)

Cluster A & HB+ patients
N = 22 (21 Death)
Cluster Analysis of all human HCC (139 patients)
Expression of Hepatic Oval Cell Marker Genes in human HCC
Comparison of Proliferation and Apoptosis Indices among Subtypes of HCC

A

- Cluster A & HC
  - N = 35 (24 Death)
  - Probability

- Cluster A & HB
  - N = 22 (21 Death)

- Survival (Months)

- P = 0.006

B

- Subclass A & HB
- Subclass A & HC
- Subclass B

- Proliferation index

- Apoptosis index

- n = 9
- n = 6
- n = 9

- P < 0.001
- P < 0.001
- P = 0.84
- P = 0.56
- P < 0.001
Progenitor Cell Gene Expression Signature of HCC

Differentially Expression genes between cluster A vs. B between HB vs. HC (p < 0.001)
TOP 10 Gene Networks of the HB Subtype Gene Expression Signature from Ingenuity Pathway Analysis

<table>
<thead>
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<th>Network ID</th>
<th>Score*</th>
<th># of genes in list</th>
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</tbody>
</table>

* The score is a numerical value used to rank networks according to how relevant they are to the genes in input.
FOS & JUN Network in HB subtype of HCC
HB Specific Gene Expression Signature

Cluster A | Cluster B
---|---
HB | HC

HIF1A
EGLN2
Summary and Conclusions

• Cross comparison of independent gene expression signatures with human data can identify novel classes of human HCC that are homogeneous in underlying biology and clinical outcome.

• A fraction of human HCC may originate from liver stem cells (oval cells?)

• Emphasizes the importance of JUN and FOS in hepatocarcinogenesis.

• HB subtype of HCC might be more metastatic.

• Need for a new mouse model(s) for HB subtype.
Molecular Classification of HCC

Cluster Analysis
- Subclass A
- Subclass B

Cell Proliferation

Apoptosis

Stem Cell Signature
- HB
- HC

Vascular Invasion

Combined
- HB
- A
- B

Patient Survival

Oncogenic Drivers?
- JUN, FOS
- EGFR
- MYC, E2F1
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Diabetes is Associated with a Two-fold Increase in Risk of HCC

El-Serag HB, Tran T, Everhart JE, Gastroenterology 2004

No Diabetes
N= 650,620

Diabetes
N= 173,643

P<0.0001

HCC Rate (%)

Years of follow up
Genomics of Preneoplastic Lesions in Human HCC

Pal Kaposi-Novak
Pathogenesis of Human HCC

Preneoplasia Conversion HCC

- LGDN
- "Nodule in Nodule"
- RN
- DF
- HGDN

Malignant
Nodular Liver Lesions

- Cirrhotic Nodules (n=26)
- LGDN (n=3)
- HGDN (n=13)
- HCC (n=13; early <2cm)

-Samples obtained from explanted livers from 10 patients
Unsupervised Clustering

Variance of gene expression

2-fold regulated genes

Genes with >2-fold change in expression
Differentially expressed genes

**CN vs DN** (n=120; p<0.005)

**DN vs HCC** (n=559; p<0.005)
Analysis of the differentially expressed genes indicated activation of MYC regulated genes during the transition from dysplasia to carcinoma.
Identification of K-Ras expression signature in human lung adenocarcinomas
MYC, RAS, Beta-Catenin induced expression signatures identified in adenovirus transfected human breast epithelial cells.
- Validated in both mouse models and in human malignancies

MYC regulated genes identified in adenovirus transfected HUVEC cells with SAGE.
- Validated with Chip and promoter analysis

Menessens et al. 2002 PNAS 99(9): 6274
The MYC up-regulated genes are significantly enriched in the hepatocellular carcinomas.

While the RAS signature genes are not generally enriched in the hepatocellular carcinomas.
GSEA – MYC and RAS Signatures

Breast epithelial cells

MYC UP

RAS UP

MYC DOWN

RAS DOWN

Breast epithelial cells

HUVEC
Clustering with the MYC Signatures

MYC-HUVEC  MYC-Breast epithelial

[Heatmap images showing clustering patterns for MYC-HUVEC and MYC-Breast epithelial samples, with color coding for different conditions: CN, LGDN, HGDN, HCC]
Summary and Conclusions

A functional genomics approach using pathway and gene set enrichment analysis revealed frequent transcriptional activation of the MYC target genes during the dysplastic to malignant conversion.

Frequently up-regulation of MYC induced gene set in the early HCCs could indicate a potentially decisive role for MYC and for the MYC regulated gene set and in malignant cell proliferation and tumor progression.
Key “Players”

Diego

Valentina

Liz

Ju-Seog

Pal

Cedric
Epidemiology of Hepatitis B

Prevalence of HBsAg Carrier State

WHO

- >8%
- 2-8%
- <2%
HCV - Epidemiology

**Prevalence**

**Worldwide**
- 170 million
- (3%)

**United States**
- Anti-HCV positive: 3.9 million
  - (1.8%)
- HCV RNA positive: 2.7 million
  - (1.4%)

*Alter MJ et al., New Engl J Med 1999*