Genomics of Hepatocellular Carcinoma: Implications for Pathogenesis and Treatment

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Topics

Introduction

Expression Signatures
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

(El-Serag, HB; Personal Communication)
Integrative Functional Genomics of Human HCC

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

Integration of gene expression data
Supervised and unsupervised analysis of data

- 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

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
- ONCOCENES
Oligo/cDNA Microarray

Normal Livers (n=19)

Oligo microarrays (70-mer probes for 21,320 genes)
Survival Analysis of Two Clusters

Cluster A
Cluster B

Survival (months)

Probability

p = 2.56 x 10^{-5}

Cluster A
N = 39 (32 Death)

Cluster B
N = 49 (19 Death)
Survival Genes (SG406)

406 genes

Cluster A
Cluster B

HB
HA

p = 8.46 x 10^{-4}

BOTTOM 50 percentile
N = 44 (15 Death)

TOP 50 percentile
N = 44 (37 Death)
Functional Analysis of Selective Survival Genes

- Cell Cycle Regulation and Cell Proliferation
  CDK4, TOPBP1, CCNB1, PCNA, KNTC1, MAPRE1, NPM1, MCM7

- Ubiquitination and Sumoylation
  UBE2O1, USP1, UBA2, RBX1, SMT3H2

- Histones
  HIST2H4, HRMT1L2, CRFG, HIST1H4C

- Apoptosis
  PTMA, SET, YWHAB, YWHAH, YWHAQ, PDCD5
Controlling the Caspase Cascade

Modified from
Science,299,214,2003
Stem Cells and Liver Cancer
Normal StemCell

Embryonic precursor

Self-renewal

Normal fetal stem cell

Self-renewal

Normal adult stem cell

Differentiation

Mature Cells
(limited proliferative potential)

Cancer StemCell

Normal stem cell

Self-renewal

Mutagenesis

Restricted progenitor

Premalignant stem cell

Self-renewal

Malignant cancer stem cell

Restricted Differentiation

"Benign" Cancer
(limited proliferative potential)
Comparative Functional Genomic Approach
Cellular Origins of Cancer

- Cancer cells evolve from normal cells following accumulation of genetic and epigenetic alterations.

- Gene expression patterns in cancer cells fatefully reflect these alterations. However, significant fraction of gene expression program in the cancer cell retains the characteristics of the original normal cellular lineages (Nature 2000, 403:503-511).

Three independent data sets

Human HCC: N = 139
   • Chinese HCC: 61
   • Caucasian HCC: 78

Mouse HCC from 5 models: N = 39
   • DENA-induced (3 tumors)
   • Myc (8 tumors)
   • E2f1 (10 tumors)
   • Myc/E2f1 (9 tumors)
   • Myc/Tgfa (9 tumors)

Rat normal liver cells: N = 9
   • Hepatoblasts (4)
   • Hepatocytes (5)

511 genes were selected to have dynamic range of changes in expression during liver development
Genes Shared Between Human and Mouse HCC Gene List and Rat Hepatoblast Gene List

- 80 genes

Diagram with Venn diagrams showing overlaps between Rat, Mouse, and Human (HCC, China) with 80 genes in common.
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

P < 0.001

Survival (Month)

Probability

HC patients
N = 47 (30 Death)

HB patients
N = 14 (13 Death)
Class Prediction of human HCC with hepatoblast gene expression signature

Training Set (n=61) Chinese HCC

Test Set (n=78) Caucasian HCC

Selection of differentially expressed genes between subtypes

Development and training of prediction models (6 classifiers)
LDA, 1NN, 3NN, NC, SVM, CCP

Leave-one-out cross validation

Evaluation of predictive value by cross comparison of predicted outcomes and Kaplan-Meier plots
Predicted Outcomes of HCC with Hepatoblast Gene Expression Signature
Class Prediction of Human HCC with Hepatoblast Gene Expression Signature

HCC Patients (n = 139)

Training (n = 61)

Chines HCC

Integration of data and cluster analysis

Survival 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)
- KNN (K-Nearest Neighbor)
- CCP (Compound Covariate Predictor)

Test (n = 78)

Caucasian HCC

Kaplan-Meier plot for Chines HCC (n = 51)

p = 1.5 x 10^-5

HB- patients N = 47 (3D Death)

HB+ patients N = 14 (13 Death)

Kaplan-Meier plot for Caucasian HCC (n = 52)

p = 4.7 x 10^-5

HB- patients N = 44 (23 Death)

HB+ patients N = 8 (8 Death)

HCC (China)

HCC (Belgium, Mayo)
Cluster Analysis of all human HCC (N = 139)
Expression of Hepatic Oval Cell Marker Genes in human HCC

Gene Expression ratios (log2)

- HB subtype in Cluster A
- HC subtype in Cluster A
- Cluster B
- Surrounding Tissues

Legend:
- AFP
- KRT19
- KRT7
- VIM
Comparison of Proliferation and Apoptosis Indices among Subtypes of HCC

Comparison of Proliferation and Apoptosis Indices among Subtypes of HCC

- Proliferation index
  - Subclass A & HB: n = 9
  - Subclass A & HC: n = 6
  - Subclass B: n = 9

- Apoptosis index
  - Subclass A & HB: n = 9
  - Subclass A & HC: n = 6
  - Subclass B: n = 9

*P < 0.001*

*P = 0.84*

*P = 0.56*

*P < 0.001*

*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)

- Cluster A
- Cluster B
- Hepatoblast
- Hepatocyte

Cluster A vs. B (5202)
HB vs. HC (3273)
**TOP 10 Gene Networks of Progenitor Cell Gene Expression Signature from Ingenuity Pathway Analysis**

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<thead>
<tr>
<th>Rank</th>
<th>Score</th>
<th>Genes in Network</th>
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*The score is a numerical value used to rank networks according to how relevant they are to the genes in input dataset (HPC genes). The score takes into account the number of genes in the network and the size of the network to approximate how relevant this network is to input gene list.*

### Differentially Expression genes between H8 vs. HC (p < 0.001)

907 genes

### TOP 10 List of Gene Networks from Ingenuity Pathway Analysis

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FOS & JUN Network in HB subtype of HCC
AP-1 Down-Stream Targets

MMP1, PLAUR, TIMP1, CD44, VIL2(EZrin)
All Subclasses of Human HCC

Survival

Recurrence

P < 0.001 (n=113)

P < 0.005 (n=66)
Molecular Classification of HCC

Cluster Analysis

- Subclass A
- Subclass B

Cluster Analysis Metrics:
- Cell Proliferation
- Apoptosis

“Stem Cell Signature”
- HB
- HCC(A+B)
- Vascular Invasion

Combined
- HB
- A
- B

Combined Metrics:
- Patient Survival
Comparative Functional Genomics to Define Gene Expression Signatures
Objectives

• To identify HGF/c-Met regulated target genes in primary hepatocytes with oligonucleotide microarray analysis

• Use the c-Met pathway specific expression signature to identify subgroup of human HCC
HGF and c-Met

Morphogenesis-Mitogenesis-Motogenesis

Embryonic Development
- liver
- brain
- placenta
- muscle
- diaphragm

Tissue Regeneration
- kidney
- Liver
- mammary glands
- neuron

Tumor & Metastasis
- c-met activation can be tumorigenic
- important role during metastasis
Importance of HGF/c-met Signaling

Both HGF and c-met knockouts are embryonic lethal.
Liver-Specific c-Met Knockout

- To generate MetLivKO mice, c-met<sup>fl/fl</sup> mice were crossed with AlbCre transgenic mice.
- No detectable level of c-met deletion in the non-hepatic tissues.
- WB analysis revealed the p170 precursor with trace amounts of the mature p140 form of c-Met.
- c-Met-dependent signaling via p42/p44 and AKT and HGF growth stimulation were abolished in c-Met-/- hepatocytes.

**Western blot**

- Cre-Ctl MetLivKO Hepa1-6
- Met - p170 - p140
- GAPDH

**c-Met IHC**

- +/+ -/-

**c-Met signaling**

- Cre-Ctl MetLivKO (min after HGF)
- P-p42/p44
- p42/p44
- P-AKT
- AKT

**HGF growth stimulation**

- wt MetLivKO
- Arbitrary units
- Hours after HGF

Conditional deletion of exon 16 inactivated the c-met gene.
Experimental Design

O/N serum free 0h 0.5h 2h 12h 24h

Alb-Cre +/- c-met fl+/fl+

Alb-Cre +/+-

- HGF

Array analysis
Analysis of Microarray Data

Non-parametric t-test (1.5-fold diff, p<0.001, 1000 random permutations FDR<10%)

730 differentially expressed genes, with HGF/c-Met dependent expression profiles

Permanent differences

Inducible differences
HGF/c-Met Induced Genes

Early Up-regulated

Early and Late Up-regulated

Late Up-regulated

Early Down-regulated

Early and Late Down-regulated

Late Down-regulated
Functional Analysis of Target Genes

- **Angiogenesis**: Vcam1, Angplt4, Angptl3, CD63, Ctgf, Neo1, Robo1, Anax2
- **Cell motility**: Opn, Cap1, Nck2, Arpc1b, Hsp5a, Msn, Mmp7, Cxcl2, Capn2, Intga3, Igfb1, IntgaV
- **Cytoskeleton**: Tuba1, Tubb3, Tubb6, Krt2-8
- **Cell adhesion**: Cldn2, Cdh17, Fath, Zo-3
- **Apoptosis regulation**: Pea15, Pps, Moap, Bak, Fas, Tnsfr23
- **Oxidative and xenobiotic stress response**: Nrf2, MafF, MafK, Gclc, Gsta1, Gsta3, Gstm2, Gstm6, Gstt1, Aldh1a1, Aldh1a7, Adh1, Ephx2
- **Lipid metabolism**: Glyat, Acox1, Lipc, Dgat2, Cyp4a10, Fasn, Fabp1
Comparison with Human HCC Datasets

Mouse hepatocytes

LEC set

Stanford set

730 HGF/c-Met dependent genes

448 genes

303 genes

202 genes

142 HCC samples

103 HCC + 7 metas. samples
Cluster Analysis with Stanford Dataset
Cluster Analysis with LEC Dataset
HGF/c-Met Signature Associated with Aggressive Phenotype

**Vascular Invasion Rate**

- Chi sq < 0.05

**Microvessel Density**

- High c-Met activation
- Low c-Met activation

**CD 34**

- High c-Met activation
- Low c-Met activation

**c-Met**

- High c-Met activation
- Low c-Met activation
HGF/c-met Signature Identifies Patients with Poor Prognosis
Integrative Functional Genomics of Human HCC

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

Integration of gene expression data
Supervised and unsupervised analysis of data

HCC Mouse Models
- C3H/10T1/2
- Denovo
- Albino
- C57

Human HCC
- Subclass A
- Subclass B

HCC Cell Lines

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

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
Acknowledgment

LEC

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Dr. Kirstin Meyer
Dr. Lewis Roberts
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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

Years of follow up

HCC Rate (%)

0 2 4 6 8 10 12 14