




약 력

1. 인적사항

| | | |
|---|------|--------------------|
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3. 주요연구실적(개조식, 간단하게)

- 박사과정 ; - Mouse t-complex의 embryonic lethal gene인 tcl-w5의 cloning 연구
- Postdoctorate ; - Mammalian embryonic transcription factor인 Oct-4의 발현조절기작 연구
- 한국생명공학연구원 ; - 동물형질전환에 의한 질환모델동물 개발 및 유전자기능 연구
 - 암 유전자치료 기술 연구
 - DNA chip 및 cell-based assay system을 이용한 암 관련 유전자 발굴 및 기능 연구

Novel target genes of hepatocellular carcinoma identified by chip-based functional genomic approaches

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Soo Jin Lim, Mi Na Kim, Sang Mi Han, Ye Jin Jang, Suk Jin Yang,
Haiyong Jung, Sang Soon Byun, Jeong Ju Lee, Jung Hwa Oh,
Kyung Chan Park, Soo Jung Kim, Sangsoo Kim, Nam Soon Kim,
Yong Sung Kim, Hyang Sook Yoo, Young Il Yeom**

*Functional Genomics Research Center,
Korea Research Institute of Bioscience & Biotechnology*

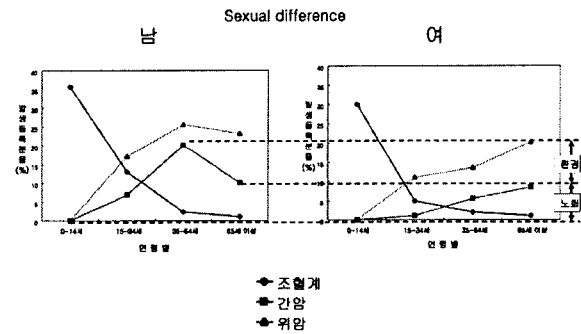
Cellular functions are carried out by a concerted action of biochemical pathways whose components have genetic interactions. Abnormalities in the activity of the genes that constitute or modulate these pathways frequently have oncogenic implications. Therefore, identifying the upstream regulatory genes for major biochemical pathways and defining their roles in carcinogenesis can have important consequences in establishing an effective target-oriented antitumor strategy. We have analyzed the gene expression profiles of human liver cancer samples using cDNA microarray chips enriched in liver- and/or stomach-expressed cDNA elements, and identified groups of genes that can tell tumors from non-tumors or normal liver, or classify tumors according to clinical parameters such as tumor grade, age, and inflammation grade. We also set up a high-throughput cell-based assay system (cell chip) that can monitor the activity of major biochemical pathways through a reporter assay. Then, we applied the cell chip platform for the analysis of the HCC-associated genes discovered from transcriptome profiling, and found a number of cancer marker genes having a potential of modulating the activity of cancer-related biochemical pathways such as E2F, TCF, p53, Stat, Smad, AP-1, c-Myc, HIF and NF- κ B. Some of these marker genes were previously known to modulate these pathways, while most of the others not. Upon a fast-track phenotype analysis, a subset of the genes showed increased colony forming abilities in soft agar and altered cell morphology or adherence characteristics in the presence of purified matrix proteins. We are currently analyzing these selected marker genes in more detail for their effects on various biological processes and for possible clinical roles in liver cancer development.

Novel target genes of hepatocellular carcinoma identified by chip-based functional genomic approaches

2006. 2. 16.

한국생명공학연구원
염영일

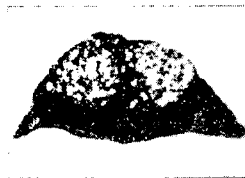
한국인 주요 암 발생을 비교 (2002년)



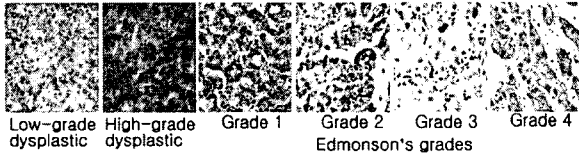
Various types of liver cancers



Capsule formation

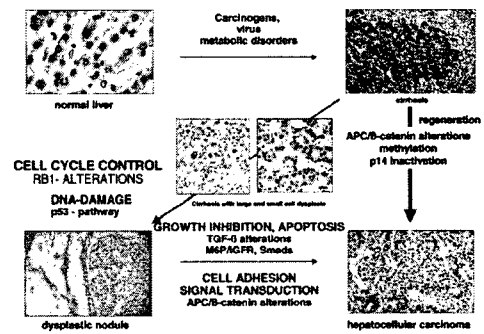


Infiltrative nodular



Low-grade dysplastic High-grade dysplastic Grade 1 Grade 2 Grade 3 Grade 4 Edmonson's grades

Multistage process of hepatocarcinogenesis



Tannapfel A., *Virchows Arch* (2002) 440:345-52

Liver cancer samples: Clinicopathological profiles

- Paired non-tumor (cirrhosis) - tumor samples
- Dr. Jong Young Choi (Catholic Medical Center)

| Age | | | Edmonson's grade | | | | Etiology | | | | Intrahepatic Invasion | | Portal vein thrombosis | |
|-----|-------|-----|------------------|----|-----|----|----------|---|-----|------|-----------------------|----|------------------------|----|
| -49 | 50-59 | 60- | I | II | III | IV | B | C | Alc | NBNC | Yes | No | Yes | No |
| 12 | 14 | 13 | 11 | 10 | 10 | 10 | 30 | 3 | 4 | 4 | 10 | 29 | 9 | 31 |

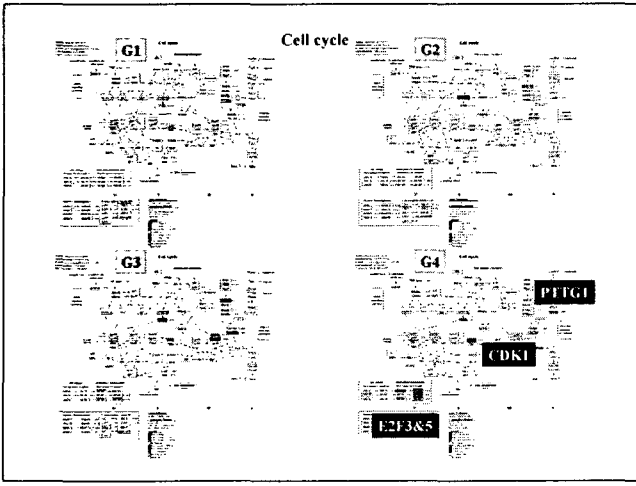
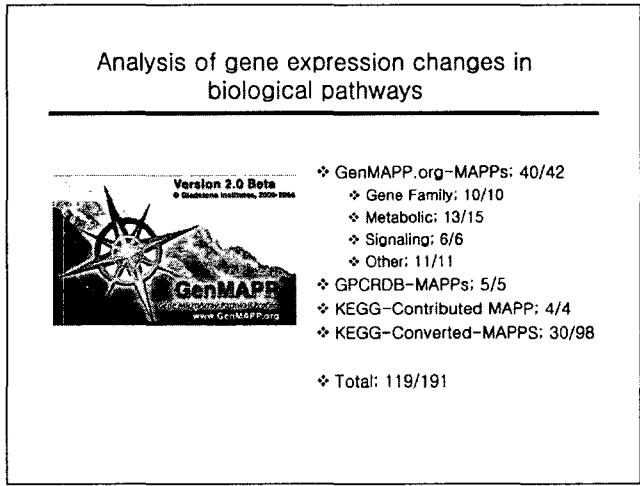
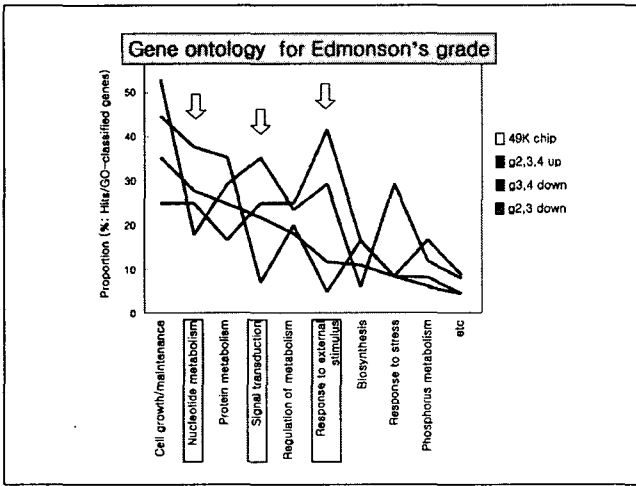
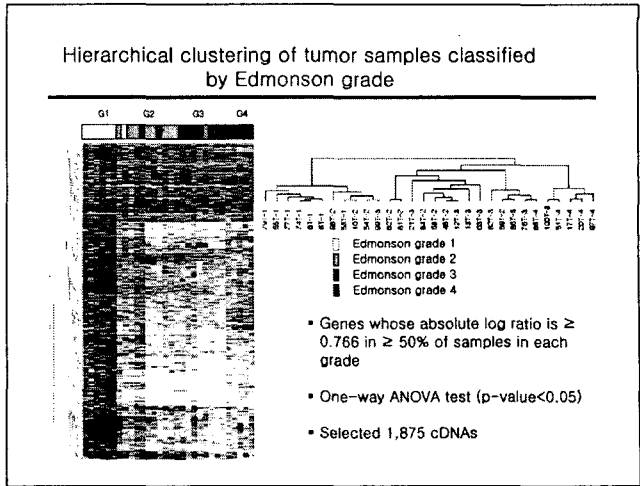
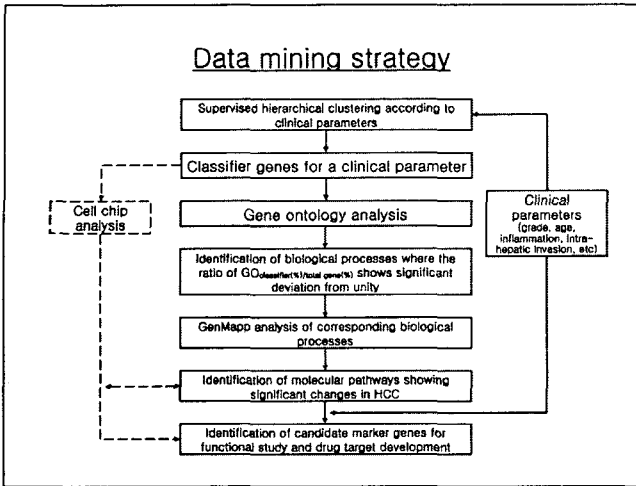
| Recurrence | | Tumor number | | | Inflammation | | α-Fetoprotein (ng/ml) | | | | Survival | |
|------------|----|--------------|---|----|--------------|----|-----------------------|-------|---------|------|----------|----|
| Yes | No | 1 | 2 | 3- | 1 | 2 | <19 | 20-98 | 100-399 | 400- | Yes | No |
| 13 | 27 | 24 | 8 | 6 | 26 | 12 | 15 | 7 | 5 | 14 | 27 | 12 |

□ Significant associations

| Parameter 1 | Parameter 2 | p-value |
|------------------------|------------------------|---------|
| Edmonson grade | Tumor size | 0.017 |
| Recurrence | Survival | 0.029 |
| | Tumor size | 0.028 |
| Tumor size | AFP | 0.025 |
| | AFP | 0.035 |
| Nodule number | Intra-hepatic invasion | 0.022 |
| | Albumin | 0.01 |
| Intra-hepatic invasion | Portal vein thrombosis | 0.024 |
| Portal vein thrombosis | Inflammation | 0.013 |
| Inflammation | Albumin | 0.016 |

□ Non-significant associations

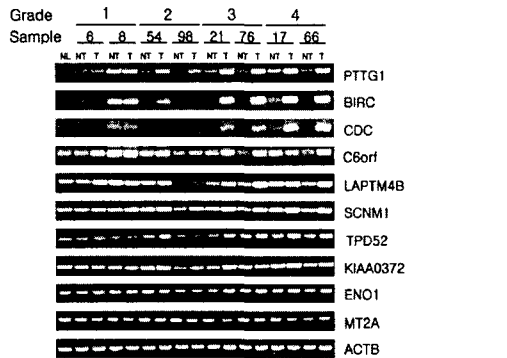
| Parameter 1 | Parameter 2 | p-value |
|----------------|------------------------|---------|
| Edmonson grade | Recurrence | 0.145 |
| | Survival | 0.664 |
| Recurrence | Intra-hepatic invasion | 0.141 |
| | Intra-hepatic invasion | 0.171 |
| Survival | Size | 0.884 |
| | Intra-hepatic invasion | 0.412 |
| | Portal vein thrombosis | 0.215 |



Pathways showing significantly altered structure in HCC: GenMAPP analysis of Edmonson's grade classifiers

| Regulation | Pathway structure | Edmonson's grade |
|------------|---|------------------|
| Up | Cell cycle | in G2-G4 tumors |
| | Nucleotide metabolism | in G4 tumors |
| | Cytoplasmic tRNA synthesis | in G4 tumors |
| Down | Blood clotting | in G2-G4 tumors |
| | Bile acid biosynthesis | in G2-G4 tumors |
| | Fatty acid metabolism | in G2-G4 tumors |
| | Fructose and mannose metabolism | in G2-G4 tumors |
| | Glycolysis and gluconeogenesis | in G2-G4 tumors |
| | Inflammatory response pathway | in G2-G4 tumors |
| | Nitrogen metabolism | in G2-G4 tumors |
| | Starch and sucrose metabolism | in G2-G4 tumors |
| | Urea cycle and metabolism of amino groups | in G2-G4 tumors |

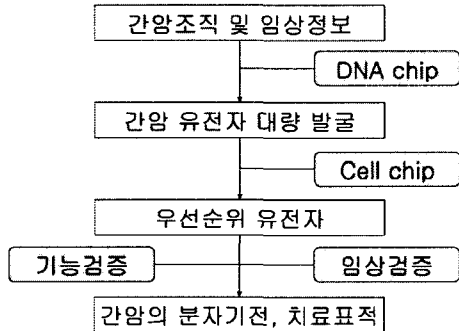
Validation of expression patterns by RT-PCR



Clinical parameters showing statistically significant classifier genes

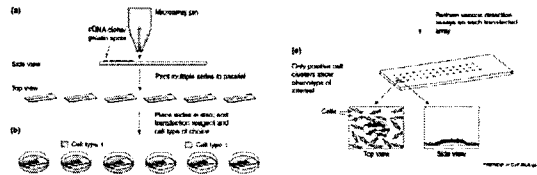
| Clinical parameter | # of cDNA | |
|-----------------------|---------------|----------------|
| | Nontumor | Tumor |
| Tumor grade | 194 (p<0.05) | 1604 (p<0.005) |
| Age | 37 (p<0.01) | 170 (p<0.01) |
| Cause | 715 (p<0.001) | 390 (p<0.005) |
| Inflammation | 75 (p<0.001) | 221 (p<0.01) |
| Intrahepatic invasion | 91 (p<0.01) | 156 (p<0.01) |
| Recurrence | 114 (p<0.01) | 250 (p<0.001) |
| AFP | - | 137 (p<0.005) |
| PVT | - | 125 (p<0.01) |
| Survival | - | 313 (p<0.005) |

Genomic approaches for the molecular dissection of liver cancer

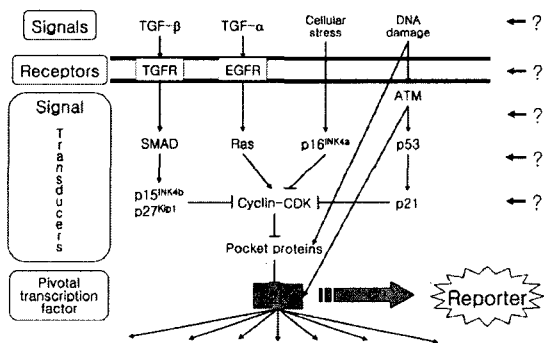


Reverse transfection을 응용한 cell microarray

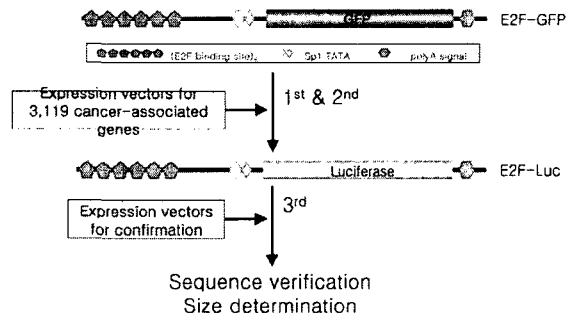
- Ziauddin, J. and Sabatini, D.M. Nature, 411:107-110(2001)
- Spotted arrays of plasmid for cDNA or siRNA expression or of oligonucleotide
- Immobilization of plasmid DNA or oligonucleotide in a gel
- Gene transfer by reverse transfection followed by an assay
- GFP/RFP 등 형광 단백질을 이용한 reporter assay, 항원표지된 항체를 이용한 immunocytochemistry, RNAi를 이용한 방법, Western blot 등 다양한 방법으로 실험이 가능



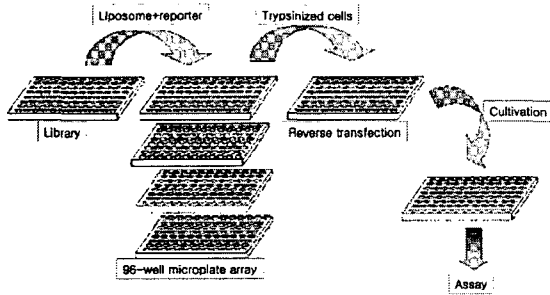
Convergence of complex intracellular signals into a pivotal transcription factor



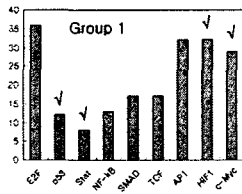
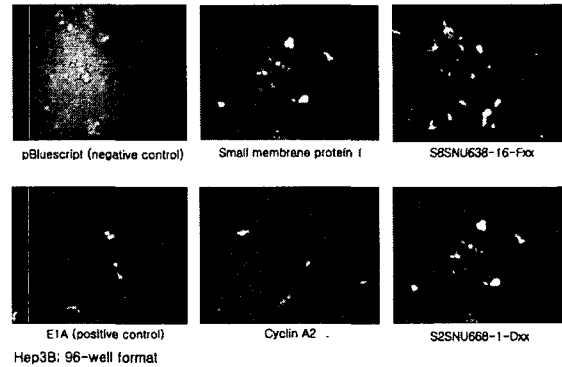
Screening of E2F modulators by reverse transfection in 96-well format



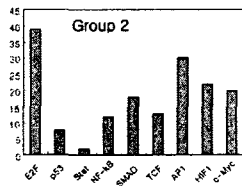
96-well microplate를 이용한 reverse transfection



E2F modulators identified by reverse transfection



- Group 1 genes
 - NT>G1>G2=G3=G4
 - Tumor suppressive or oncogenic
 - pro- or anti-growth
 - pro- or anti-apoptosis
 - Tumor initiation



- Group 2 genes
 - NT<G1<G2=G3=G4
 - Oncogenic
 - promotion of growth & proliferation
 - anti-apoptosis
 - Tumor progression

Representative Group 1 Genes (NT>G1>G2=G3=G4)

- CASP1
- GADD45B & G
 - These genes are often coordinately expressed and can function cooperatively in inhibiting cell growth.
- BIRC2
 - Suppression of apoptosis in mammalian cells by NAIP and a related family of IAP genes. Nature 379, 349(1996)
- ID2
 - Id2 is critical for cellular proliferation and is the oncogenic effector of N-myc in human neuroblastoma. Cancer Res. 62, 301(2002)
 - Id2 is a retinoblastoma protein target and mediates signalling by Myc oncoproteins. Nature 407, 592(2000)
- LRDD
 - The PIDDosome, a protein complex implicated in activation of caspase-2 in response to genotoxic stress. Science 304, 843(2004)
- YWHAE
 - 14-3-3 proteins associate with cdc25 phosphatases. Proc. Natl. Acad. Sci. U.S.A. 92, 7892(1995)
 - Is probably a multifunctional regulator of the cell signaling processes mediated by CAM kinase II and PKC.

Representative Group 2 Genes (NT<G1<G2=G3=G4)

- Fos, Jun-B, Shc1, H-Ras, PCNA
- PIK1
 - The molecular basis for phosphodependent substrate targeting and regulation of Plks by the Polo-box domain. Cell 115, 83(2003)
- PTTG1
 - Human securin interacts with p53 and modulates p53-mediated transcriptional activity and apoptosis. Nat. Genet. 32, 306(2002)
- MDK
 - Midkine binds to anaplastic lymphoma kinase (ALK) and acts as a growth factor for different cell types. J. Biol. Chem. 277, 35990(2002)
- CDK5
 - Cdk5 is a key factor in tau aggregation and tangle formation in vivo. Neuron 38, 555(2003)
- RBBP7
 - A cellular protein that competes with SV40 T antigen for binding to the retinoblastoma gene product. Nature 350, 160(1991)

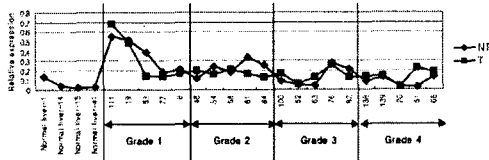
Prioritization of pathway modulators for drug targets



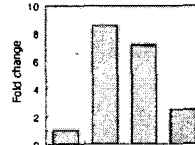
- 3,119 ← Expression profiling of tumor tissues
- 682 ← Cell chip analysis
- 80 ← Expression in cancer
 - Association with clinical parameters
 - Cell-based phenotype
 - Target gene expression
 - Strong cell chip response
- 10 ← Extensive analyses at molecular, cellular, organismal and clinical levels
 - Over-expression, siRNA, Ab, xenograft

WAOH (Wnt-activating oncogene of HCC)

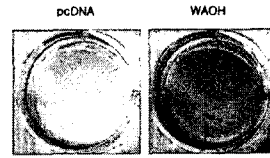
1. Protein kinase activity
2. ATP binding
3. Frequent up-regulation in HCC



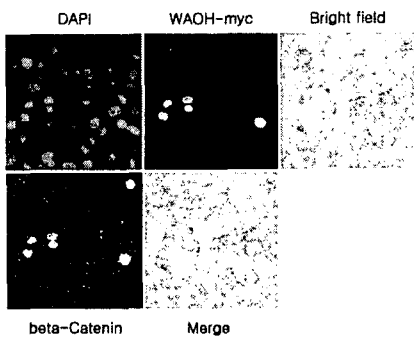
TCF-dependent transcription



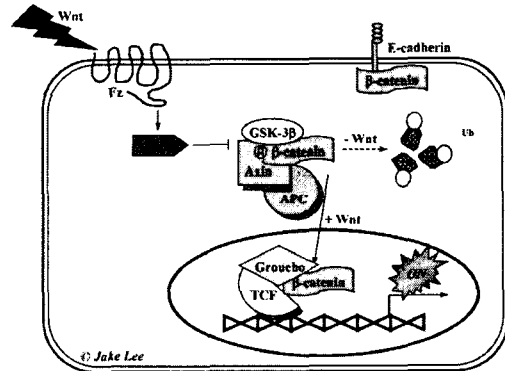
Colony formation in soft agar



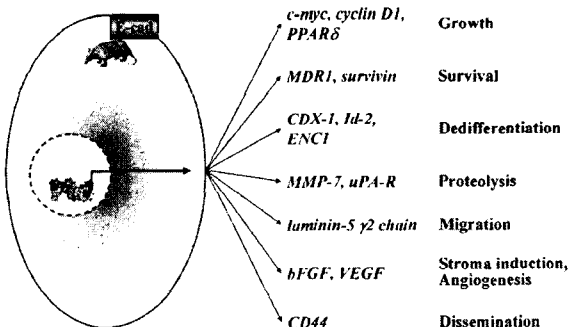
| Condition | MOCK | m.Beta-cal | WAOH | sRNA |
|-----------|------|------------|------|------|
| + | - | - | - | - |
| - | + | + | + | + |



Canonical Components of the Wnt/ β -catenin Signaling



The oncogenic potential of nuclear β -catenin deduced from known target genes



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|-----------------|---------|------|
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