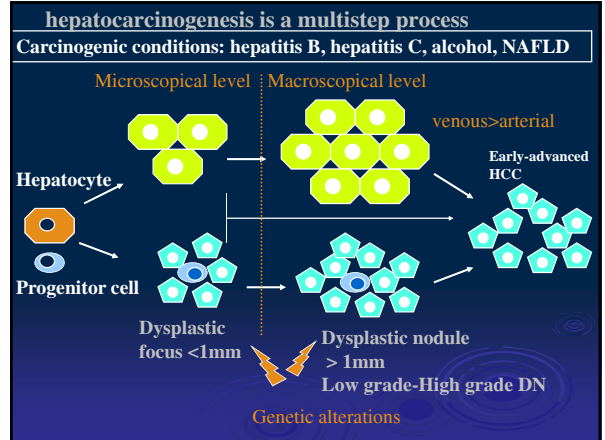




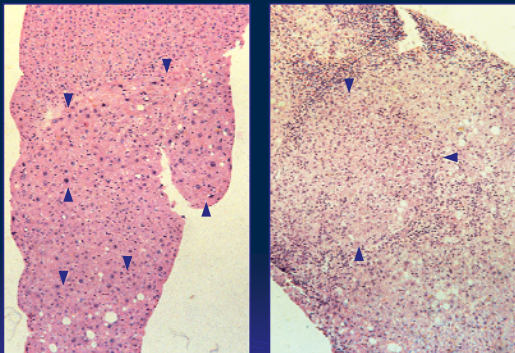
# Histopathology and molecular classification

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Belgium

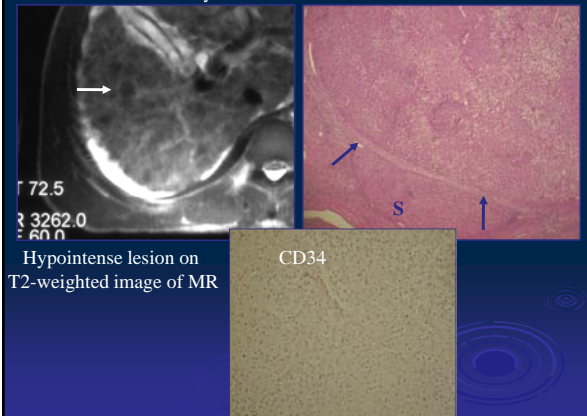


Needle liver biopsies of 115 chronic hepatitis pts; 10 yrs fu



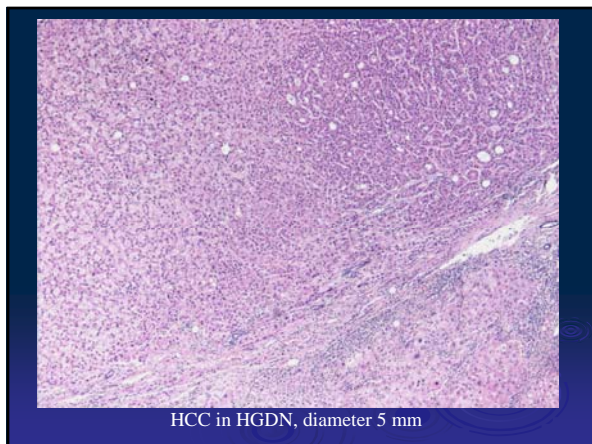
Libbrecht et al Histopathology 2001

Early cancer with a diameter of 10 mm



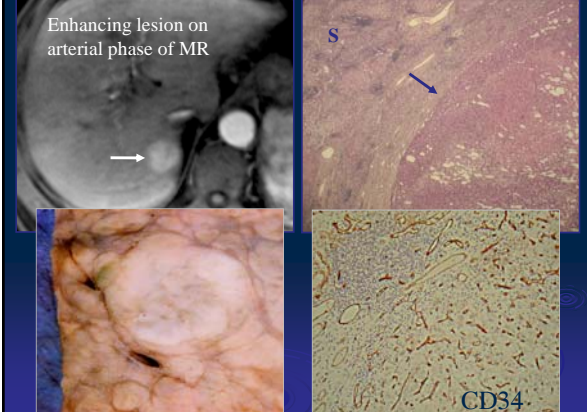
Hypointense lesion on T2-weighted image of MR

CD34



HCC in HGDN, diameter 5 mm

HCC with a diameter of 20 mm



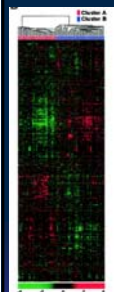
Enhancing lesion on arterial phase of MR

CD34



## Glypican-3 IH: sensitivity 77%, specificity 96%

Capurro et al Gastroenterology 03, Libbrecht et al Am J Surg Pathol 06

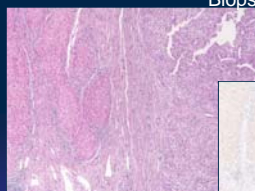


Panel with HSP70 and GS

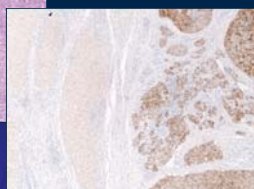
Di Tomasso et al Hepatology 07

DD High grade dysplastic nodules vs early carcinoma < 2.5cm

Biopsies included



Glypican-3



## A Molecular Signature to Discriminate Dysplastic Nodules from Early Hepatocellular Carcinoma in HCV-Cirrhosis

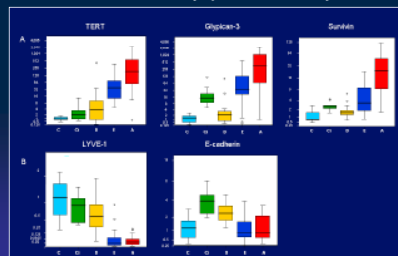
Josep M. Llovet, et al Gastroenterology 2007

### Quantitative real-time PCR analysis of 5 genes

3 up-regulated: **TERT, glypican 3, survivin**

2 down-regulated: **LYVE-1 E-cadherin**

allows to discriminate between dysplasia and early HCC



## Need for good reproducible and prognostically significant criteria

- DD dysplastic nodules versus early cancer
- **Established cancer: prognostically significant classification**
- HCC vs CC **Too Simple!!**

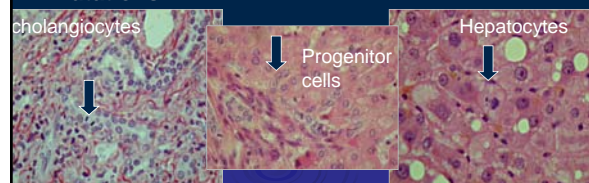
## HCCs are derived from a single cell (monoclonal)

Which cell?

- longevity
- high self-renewal capacity

HEPATOCELLULAR CARCINOMA

Necessary to accumulate mutations



## HPC in Chronic Liver Diseases



Telomere shortening and replicative senescence of mature hepatocytes (and not of hepatic stellate cells or lymphocytes) is a general feature of the **cirrhotic** stage of a variety of chronic liver diseases

Wiemann FASEB J 03, Falkowski J Hepatol 03, Rudolph Science 00, Fausto Hepatology 2004

**This inhibition of replication is associated with progenitor cell activation in human liver diseases**

De Vos Am J Pathol 92, Roskams J Hepatol 98, Lowes Am J Pathol 99, Libbrecht J Pathol 00, Roskams Am J Pathol 03, Katoonizadeh Liver Int 07

Since **progenitor cells** are activated in chronic liver diseases, they also form a **target cell population for carcinogenesis**



Tumor progression is driven by cancer stem cells

" Cancer Stem Cell " was defined 30 years ago ( 1977 ) as :

" tumor stem cells are the cell renewal source of a neoplasm and also serve as the seeds of metastatic spread of cancer "

although they constitute only a small minority population !

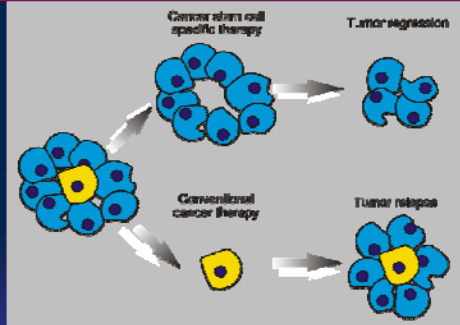
Hamburger and Salmon  
Primary bioassay of human tumor stem cells  
Science, 1977.

### Properties shared by normal stem cells and cancer stem cells

1. capacity for self-renewal
2. ability of multilineage differentiation  
*heterogeneous offspring*
3. active telomerase expression  
*longevity*
4. activation of anti-apoptotic pathways  
*longevity*
5. increased membrane transporter activity  
*drug and toxin resistance*
6. ability to migrate and metastasize  
*anchorage independence : anoikis*

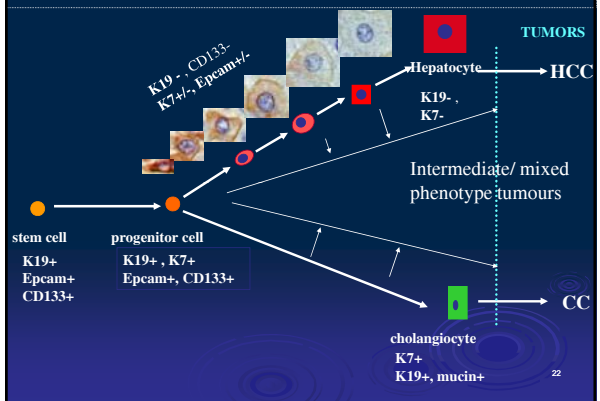
Wicha M S, Liu S, Dontu G: Cancer Stem Cells: an old Idea - a Paradigm Shift. Cancer Res, 66, 1883 - 1896, 2006.

### Therapeutic implications of Cancer Stem Cells

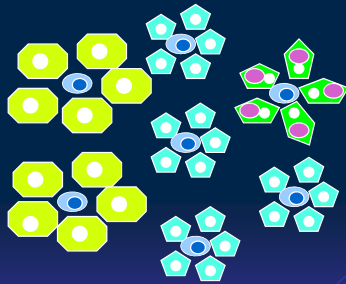


• Most therapies fail to consider the difference in drug sensitivities of cancer stem cells compared to their non-tumorigenic progeny.

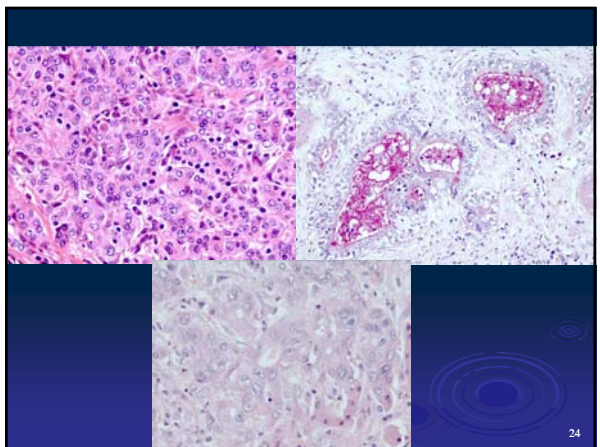
### Progenitor Cells and Intermediate Hepatocytes



### Cancer stem cells and their progeny



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### 15-16% HCC: progenitor cell features/K19+

Durnez A et al Histopathology 2006  
Group Llovet ILCA 08: 15%

### EXPERIMENTAL SETUP

**Laser Microdissection**

Non-neoplastic HPC/hepatocytes    CLC chol    CLC hep

CK19 neg and CK19 pos HCC

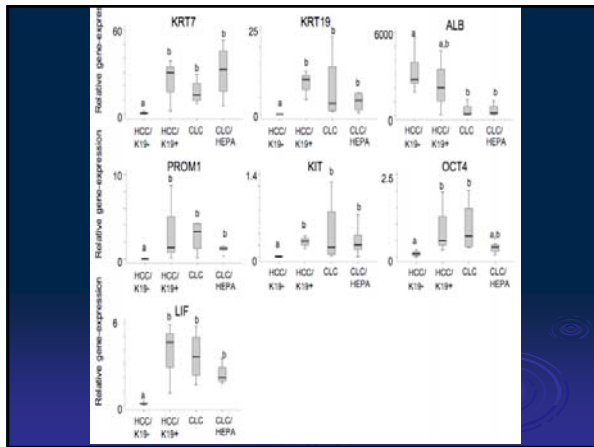
**RNA isolation**

**RNA amplification**

**Gene-expression analysis**  
- Superarray (real-time PCR 84 genes)

**IHC confirmation**

Mina Komuta, Bart Spee et al Hepatol 08



### Keratin 19 Positive Hepatocellular Carcinoma

**Survival**

Tumor-free survival rate (%)

— K19- (n=142)  
— K19+ (n=15)

(Years after surgery)

Uemishi, T. et al. Cancer Sci. (2003)

**Recurrence**

Recurrence-free rate (%)

— K19- (n=27)  
— K19+ (n=4)

P=0.009

Durnez, A. et al. Histopathology (2006)

AIM : further investigate the clinicopathological correlates and relevance of KRT19 expression at mRNA level in an extended series of human HCC

### Prognostic relevance KRT19 expression

**Postoperative Recurrence**

— Cum. Recurrence (KRT19-, n=57)  
— Cum. Recurrence (KRT19+, n=9)  
+ Sensor Times (KRT19-)  
+ Sensor Times (KRT19+)

Time, days

• KRT19 RNA (continuous variable) correlated significantly with:  
- tumor recurrence (p=0.0001, HR:1.56)

### Prognostic relevance KRT19 expression

**Postoperative Survival**

— Cum. Survival (KRT19-, n=57)  
— Cum. Survival (KRT19+, n=9)  
+ Sensor times (KRT19-)  
+ Sensor times (KRT19+)

Time, days

• KRT19 RNA (continuous variable) correlated significantly with:  
- poor survival (p<0.0001, Hazards Ratio (HR):1.43)

## Accordance with Findings of other Groups

These results are in accordance with previous findings of other groups

- **Ding et al. : ( CHINA )**  
overexpression of **K19** correlates with HCC metastasis  
*Ding et al., Mol Cell Proteomics, 3, 73-81, 2004*
- **Robrechts C et al ( BELGIUM )**  
report of undifferentiated, highly malignant  
**progenitor cell tumor**  
*Robrechts C et al., Liver, 18, 288 – 93, 1999*
- **Wu et al. : ( CHINA )**  
in univariate analysis : HCC expressing AE1 and K19 :  
**significantly shorter survival without any treatment.**  
*Wu et al., Am J Pathol, 149(4), 1167 – 73, 1996.*
- **Uenishi et al. : ( JAPAN )**  
in univariate analysis  
HCC expressing K19 and K7 : lower tumor free survival rate after curative  
resection  
**K19 expression : independent predictor of postoperative  
recurrence.**  
*Uenishi et al., Cancer Science, 94, 851 – 857, 2003.*

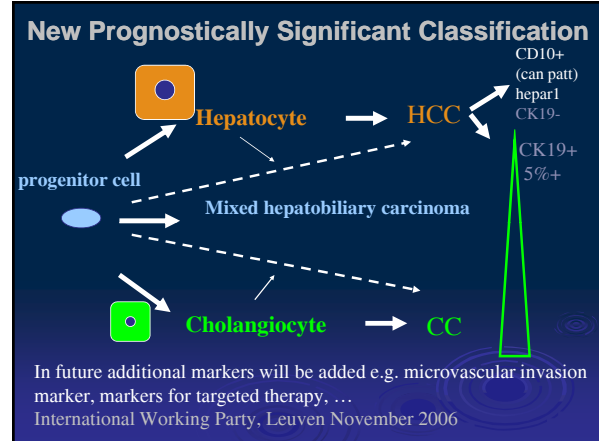
## In concordance with other groups

- **Aishima et al 2007:** 35 small HCC with biliary differentiation based on morphology, K19, mucin production, compared with 61 ordinary HCC: K19+ more extrahepatic recurrence, worse survival
- **Zhuang et al 2008:** 172 HCC with/without LN metastasis: K19 independent prognostic factor for development of LN metas and had worse survival

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**Previously identified subtypes of HCC**

**Hepatoblast signature:**  
significantly higher **KRT7**, **KRT19**,  
very bad prognosis (survival 11.9mo versus 64.4mo)  
Nature Medicine 08



**Transcriptome Classification of HCC Is Related to Gene Alterations and to New Therapeutic Targets**

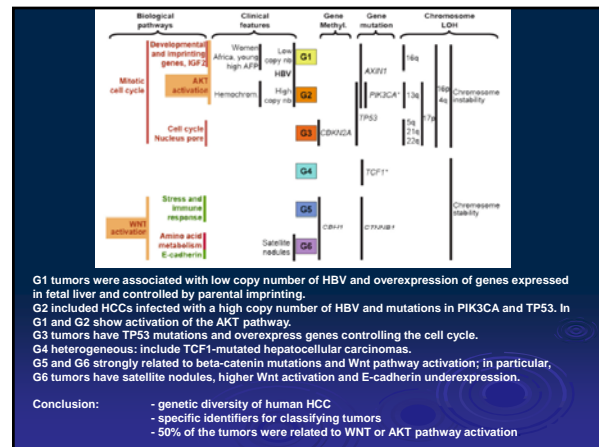
Sandrine Bruyat,<sup>1,2</sup> David S. Rickman,<sup>3\*</sup> Aurélien de Reynis,<sup>3\*</sup> Charles Rahaband,<sup>4,5</sup> Sandra Robinson,<sup>4,5</sup> Emmanuelle Jeannot,<sup>1,2</sup> Aurélie Héran,<sup>1,2</sup> Jean Saric,<sup>4</sup> Jacques Béghin,<sup>1,4</sup> Dominique Franco,<sup>1,4</sup> Paulette Bioulac-Sage,<sup>4,6</sup> Pierre Laurent-Pouig,<sup>4,6</sup> and Jessica Zucman-Rossi<sup>1,2</sup>

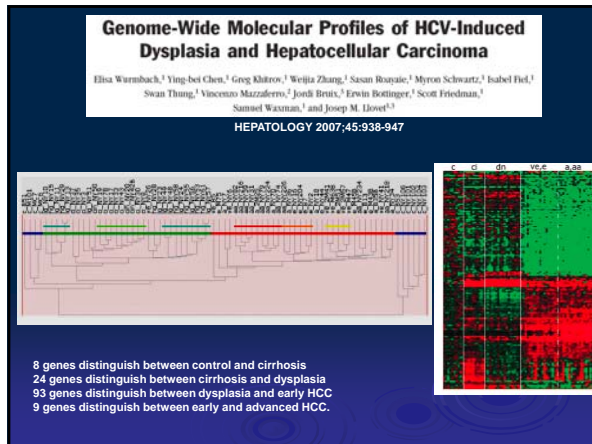
HEPATOLOGY 2007;45:42-52.

**6 homogeneous clusters**

**16 genes differentially expressed validated by quantitative real-time PCR correctly identify the 6 clusters**

Gene	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5	Gene 6
TP53	0.000	0.000	0.000	0.000	0.000	0.000
AKT1	0.000	0.000	0.000	0.000	0.000	0.000
CDKN2A	0.000	0.000	0.000	0.000	0.000	0.000
TP53	0.000	0.000	0.000	0.000	0.000	0.000
TP53	0.000	0.000	0.000	0.000	0.000	0.000
TP53	0.000	0.000	0.000	0.000	0.000	0.000
TP53	0.000	0.000	0.000	0.000	0.000	0.000
TP53	0.000	0.000	0.000	0.000	0.000	0.000
TP53	0.000	0.000	0.000	0.000	0.000	0.000
TP53	0.000	0.000	0.000	0.000	0.000	0.000
TP53	0.000	0.000	0.000	0.000	0.000	0.000
TP53	0.000	0.000	0.000	0.000	0.000	0.000
TP53	0.000	0.000	0.000	0.000	0.000	0.000
TP53	0.000	0.000	0.000	0.000	0.000	0.000
TP53	0.000	0.000	0.000	0.000	0.000	0.000
TP53	0.000	0.000	0.000	0.000	0.000	0.000





<b>Cirrhosis</b>		
Up-regulation:	- JAG1 - STAT1, CXCL9-11	(Notch receptor ligand) (Toll-like receptors)
<b>Dysplasia</b>		
Up-regulation:	- EPO, EPOR, CISH, STAT3, SOCS3 - GADD45B, GADD45G, GADD45A	(Jak/STAT pathway) (cell cycle control)
<b>Early HCC</b>		
Down-regulation:	- IFNAR1, TLR4, FOS, and CD14 - EPO, EPOR, SPRY2, and SOCS2 - BMPR2, ID2, THBS1, and DCN - FBP1, PCK1, PCK2, GYS2, FOXO1A, SOCS2	(Toll-like receptors pathway) (Jak/STAT pathway) (TGF-beta pathway) (insulin signaling)
Up-regulation:	- DKK1, FZD6, FZD7, PLCB1, LEF1 - PTCH	(beta-catenin pathway) (Hedgehog pathway)
<b>Advanced HCC</b>		
Up-regulation:	- PRIM1, PRIM2 - ASPM, PTTG1, CCNB1, CDKN2C, CDKN2A	(cell proliferation) (cell cycle control)

- ### Conclusions
- Comparison of independent **gene expression signatures** of human well characterized tumours, can identify novel classes of human HCC that are homogeneous in underlying biology and clinical outcome
  - A subgroup of human HCC probably originate from liver **progenitor cells**. This subtype has bad prognosis and can be recognized by **keratin 19** immunohistochemical staining
  - The **basic genetic profile** (< cell of origin) of a tumour is present throughout the tumour, although it can look phenotypically very heterogeneous.
  - **Cancer stem cells** should be the target of newly developed therapy. Focus on the human **liver progenitor cells and their niche** in disease and cancer!

- ### Acknowledgements
- > Prof. Em. Dr. V. Desmet
  - > B. Spee
  - > S. Vander Borgh
  - > Dr. A. Katoonizadeh
  - > Dr. M. Komuta
  - > Dr. Guido CarPELLI
  - > P. Aertsen
  - > Prof. Em. Dr. Fevery
  - > Prof. Dr. Yap
  - > Prof. Dr. Van Steenberghe
  - > Prof. Dr. Nevens
  - > Prof. Dr. C. Verslype
  - > Dr. C. Cassiman
  - > Prof. Dr. J. Pirenne
  - > Dr. R. Aerts
  - > Dr. D. Monballiu
  - > Dr. Van Beckevoort
  - > Dr. D. Bielen
  - > Dr. S. Thorgeirsson
  - > Prof. Dr. C. Trautwein
  - > Dr. T. Luedde
  - > Dr. N. Berazza
  - > Prof. Dr. A. Geerts
  - > Prof. Dr. P. Jansen
  - > Prof. M. Strazzabosco
  - > Prof. L. Fabris
  - > Prof. Dr. Pinzani
  - > Prof. Dr. Rothuizen
-