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Molecular classification of HCC

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Abstract

Hepatocellular carcinoma (HCC) is the most frequent tumors derived from the malignant transformation of hepatocytes. It is well established that cancer is a disease of the genome and as in other type of solid tumors, a large number of genetic and epigenetic alterations are accumulated during hepatocarcinogenesis process. Recent developments using comprehensive genomic tools enabled to identify the molecular diversity in human HCC. Consequently, several molecular classifications have been described using different approaches and important progresses have been done particularly with the transcriptomic, genetic, chromosomal, miRNA and methylation profiling. On the whole, all these molecular classifications are related together and one of the major determinants of the identified subgroups of tumors are gene mutations found in oncogenes and tumor suppressors. However, the full understanding of the HCC molecular classification requires additional comprehensive studies using both genomic and pathway analyses. Finally, a refinement of the molecular classification of HCC taking into account the geographical and genetic diversity of the patients will be essential for an efficient design of the forthcoming personalized clinical treatments.

Key words:

Hepatocellular carcinoma, molecular classification, oncogene, tumor suppressor gene, transcriptome, miRNA

Despite our growing comprehension of the different pathways altered in hepatocellular tumors, the molecular mechanisms that lead hepatocytes to undergo transformation and give rise to a hepatocellular carcinoma are still poorly understood. As proposed by Vogelstein in overall cancer, hepatocellular tumorigenesis is a DNA disease due to the accumulation of alterations in genes that control cell cycle and cell proliferation¹ and a large number of genetic and epigenetic alterations accumulate during this process. Classically, at initiation of carcinogenesis, the different risk factors (viral infection, cirrhotic lesions, obesity, oxidative stress ...) contribute to promote the occurrence of gene alterations in hepatocytes. Then, tumor development process will select altered hepatocytes with the highest capacity to survive and proliferate. Finally, each HCC tumor results from a specific combination of several alterations modifying oncogenic pathways. These changes are both quantitative (losses and gains of chromosome segments), qualitative (point mutations) or epigenetic and numerous cases of extinctions of gene expression secondary to the hypermethylation of their promoters have been reported². Some of the observed genetic alterations are widely shared among the different tumor types; for example, mutations in CTNNB1 and TP53 genes are found in tumors developed in several different organs, as shown in the cosmic mutation database (<http://www.sanger.ac.uk/genetics/CGP/cosmic/>)³. In contrast, other alterations are found quasi-exclusively in hepatocellular tumors and this is the case for IL6ST or HNF1A mutations⁴⁻⁶. Therefore the comprehensive knowledge of repertory of genetic alterations in a tumor type and the study of the correlation between these alterations and the different clinical and histological parameters allow refining the tumor classification and the understanding of the multistep carcinogenesis process.

Recent analysis of large number of genetic and epigenetic alterations together with transcriptome and systematic pathway analyses enabled to clarify the diversity of HCC and HCA, their molecular classification and the identification of subgroups of tumors likely to be efficiently targeted by specific drugs. In this review, we will summarize the most important molecular classifications that have been described and how these classifications could be useful in clinical practice⁷⁻⁹.

1-Oncogene and tumor suppressor gene mutations in hepatocellular tumors.

Mutations activating β -catenin are found in 20 to 40% of hepatocellular carcinomas (Table 1), showing that β -catenin is the most frequently activated oncogene in

HCC by mutation^{2, 10, 11}. The WNT/ β -catenin pathway plays a key role in liver physiological phenomena, such as lineage specification, differentiation, stem cell renewal, epithelial-mesenchymal transition, zonation, proliferation, cell adhesion and liver regeneration¹²⁻¹⁹. We showed that β -catenin mutations are associated with chromosome stability and this genetic alteration occurs more frequently in patients without HBV infection^{20, 21}. In a recent study, Audard and collaborators found that β -catenin activated HCC exhibit specific features associating high differentiation with a homogeneous microtrabeculo-acinar pattern, low-grade cellular atypia, and cholestasis²². In addition, β -catenin activated HCC are frequently developed in non-cirrhotic liver in absence of usual HCC risk factor^{22, 23}. Depending of the series, β -catenin activating mutations were found to be associated with either good^{24, 25} or bad prognosis²³ and its relation with prognosis remains debated.

TP53 is the tumor suppressor the most frequently mutated in HCC (Table 2). The mutational spectrum of *TP53* gene in HCC from Qidong and Mozambique where aflatoxin B1 (AFB1) exposure level is high, revealed G->T transversion at codon 249 in more than 50% of the tumors^{26, 27}. This mutation at codon 249 of *TP53*, leading to the amino acid substitution R249S, is exceptionally found in HCC from geographical regions without AFB1 exposure. Usually, in a determined geographic area, the frequency of the R249S mutation paralleled the estimated level of AFB1 exposure, supporting the hypothesis that the carcinogen has a causative role in hepatocarcinogenesis. In western countries, where there is no exposure to AFB1, *TP53* mutations are found in approximately 20% of the HCC, without specific hotspot of mutations²⁰. Finally, no *TP53* mutations were found in benign hepatocellular tumors^{28, 29}.

During the last 20 years, several studies have searched to identify other genes mutated in hepatocellular tumors (Table 1 and 2). Apart from CTNNB1 and TP53, all the other identified genes were found rarely mutated, i.e. in less than 10 % of the HCC cases. Although most of HCC are developed in a context chronic hepatitis and cirrhosis, a small proportion result from a malignant transformation of a benign adenoma. Accordingly, IL6ST and HNF1A that are frequently mutated in adenoma (Table 3), are rarely altered in HCC (Table 1 and 2) or in other malignant tumors³⁰⁻³². In contrast, in adenoma CTNNB1 activation was shown to be associated with a higher risk of malignant transformation^{5, 33-35}. Accordingly, this gene is found rarely mutated in HCA and more frequently activated in HCC, suggesting that β -catenin activation is a common genetic determinant associated with both benign and malignant tumorigenesis in the liver.

2- Transcriptomic classification of HCC

During the last 10 years, analysis of a large number of human HCC using expression microarray techniques enabled to identify new sub-groups of tumors defined by specific deregulation of expression of gene networks. Comparisons with functional gene modification induced in animal models or cell lines allowed to characterize the nature of these networks. The first example of integrative analyzes of transcriptomic and functional was done in the Snorri Thorgeirsson's laboratory (NCI, Bethesda, USA). In 2006, by integrating gene expression data from rat fetal hepatoblasts with HCC from human and mouse models, this team identified a subgroup of HCC that may arise from hepatic progenitor cells⁸. Importantly, this subgroup of tumors shared a gene expression pattern with fetal hepatoblasts and had a poor prognosis.

In our series of HCC surgically treated in France, we performed a genome wide transcriptomic analysis of 60 tumors together with an exhaustive characterization of structural genetic alterations and clinical parameters³⁶. In this study, unsupervised transcriptomic analysis identified six robust subgroups of HCC (termed G1 to G6) associated with clinical and genetic characteristics (Figure 1). The main classification divider was the chromosome stability status. Tumors from group G1 to G3 were chromosome instable whereas tumors from G4 to G6 were chromosome stable. Indeed, tumors presenting chromosome instable phenotype demonstrated a transcriptomic profile strikingly different from chromosome stable ones (Figure 1). Chromosome instability appears as the main driver of tumor classification as previously shown in classifications based on chromosomal and genetic aberrations^{20, 21, 28, 37}. In addition, genetic alterations and pathways analyses allowed for a refined transcriptomic classification: G1-tumors were related to a low copy number of HBV and overexpression of genes expressed in fetal liver and controlled by parental imprinting; G2 included HCC infected with a high copy number of HBV, *PIK3CA* and *TP53* mutated cases; G3-tumors were *TP53* mutated without HBV infection, a frequent P16 methylation and showed overexpression of genes controlling cell-cycle; G4 was a heterogeneous subgroup of tumors including *TCF1* mutated adenomas and carcinomas; G5 and G6, were strongly related to β -catenin mutations leading to Wnt pathway activation; G6-tumors presented satellite nodules, higher activation of the Wnt pathway and a E-cadherin under-expression. This 6-group classification has clinical application regarding the development of targeted therapies for HCC because specific pathway activations, particularly AKT and Wnt pathways, are closely associated to subgroups G1-G2 and G5-G6 respectively. Therefore we identified and

validated a robust 16-gene signature to classify HCC tumors into the 6-group transcriptomic classification. This signature should be very useful to determine alterations of specific pathways and to predict putative response to targeted drugs ³⁶.

Actually several transcriptomic analyses have been reported ^{7, 8, 36, 38-45}. Successively, a large number of molecular subgroups of tumors have been identified underlining the broad diversity of HCC in human. Despite disparities among studies in term of risk factors, geographical origin, grading of the tumors, some similar subgroups of tumors have been recurrently identified. In an attempt to describe a common molecular classification, Hoshida and collaborators performed the first “biostatistical meta-analysis” of 9 different transcriptome HCC studies⁴⁵. This constitutes an important opening step to construct an international consensus defining common bases of a robust molecular classification of HCC.

3- Micro-RNA profiling in hepatocellular tumors

Micro RNAs (miRNAs) are small non-coding RNAs that regulate gene expression. Many studies show that they are implicated in essential physiological functions and particularly in tumors ⁴⁶. Specific alterations of miRNA expression have been identified directly involved in carcinogenesis. Indeed, miRNAs could act as oncogenes or tumor suppressors. In addition, some miRNAs deregulations seem to be associated to specific tumors subtypes, suggesting that they could be used as tumor biomarker. Recently, we performed microRNA (miRNA) profiling in two series of fully annotated liver tumors to uncover associations between oncogene/tumors suppressors’ mutations, clinical and pathological features ⁴⁷. Expression levels of 250 miRNAs in 46 benign and malignant hepatocellular tumors were compared to 4 normal liver samples using quantitative RT-PCR. miRNAs associated to genetic and clinical characteristics were validated in a second series of 43 liver tumor and 16 non-tumor samples. miRNAs profiling unsupervised analysis classified samples in unique clusters characterized by histological features (tumor/non-tumor; benign/malignant tumors, inflammatory adenoma and focal nodular hyperplasia), clinical characteristics (HBV infection and alcohol consumption) and oncogene/tumor suppressor gene mutations (β -catenin and HNF1 α). Our study identified and validated miR-224 over-expression in all tumors, miR-200c, miR-200, mir-21, miR-224, miR-10b and miR-222 specific deregulation in benign or malignant tumors 5 (Figure 3). Moreover miR-96 was over-expressed in HBV tumors, miR-126* down regulated in alcohol related HCC. Down-regulations of miR-107 and miR-375 were specifically associated with HNF1 α and β -catenin

gene mutations, respectively. miR-375 expression was highly correlated to that of β -catenin targeted genes as miR-107 expression was correlated to that of HNF1 α in a siRNA cell line model. Thus, strongly suggesting that β -catenin and HNF1 α could regulate miR-375 and miR-107 expression levels, respectively. All together, hepatocellular tumors may have distinct miRNAs expression fingerprint according to malignancy, risk factors and oncogene/tumor suppressor gene alterations. Dissecting these relationships provides new hypothesis to understand the functional impact of miRNAs deregulation in liver tumorigenesis and their promising use as diagnostic markers ⁴⁷.

Several other studies have also identified a relationship between miRNA deregulation and the phenotype of HCC^{48-56, 57, 58, 59-62}. Despite different clinical features of the tumors, various risk factors, techniques and strategies used to normalized data, several miRNA altered in their expression have been recurrently found in the different studies. These observations indicate that miRNA profiling may be robust biomarkers to classify tumors. Recently the expression of miR-122 ⁶³, miR-221 ⁵¹ and miR26 ⁵⁸ were found to be associated with a poor prognosis in human HCC. All together, hepatocellular tumors may have distinct miRNAs expression fingerprint according to malignancy, risk factors and oncogene/tumor suppressor gene alterations. Dissecting these relationships provides new hypothesis to understand the functional impact of miRNAs deregulation in liver tumorigenesis and their promising use as diagnostic markers.

Conclusion

Both benign and malignant hepatocellular tumors demonstrate a broad diversity at the genetic and epigenetic levels leading to robust classification closely related to clinical features and carcinogenesis pathways. These molecular classifications are closely related together. In each of them, tumor suppressor and oncogene mutations are frequently major drivers of the subclasses. Finally, classifying tumors in homogeneous sub-groups using gene signature is a promising tool to construct rational protocols with targeted therapies and to refine prognosis

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Table 1: Major oncogenes activated by mutation in HCC (excluding cell lines)

Oncogene	Protein	Mutated HCC (%)	Main refs.
CTNNB1	Catenin (cadherin-associated protein), β 1 (β -catenin)	5-50%	10, 11, 21, 23, 36, 64-67
HRAS KRAS NRAS	Ras proto-oncogene	< 3-5%	36, 68-72
IL6ST	Interleukin 6 signal transducer (gp130)	< 3%	5
PIK3CA	Phosphoinositide-3-kinase, catalytic, alpha polypeptide	< 3%	36, 73, 74
MET	Met proto-oncogene (hepatocyte growth factor receptor)	< 1-5%	75
CSF-1R	Colony stimulating factor 1 receptor (c-fms)	2 mutations	76

Table 2: Major tumor suppressor genes inactivated by mutation in HCC (excluding cell lines)

Tumor suppressors	Protein	Mutated HCC (%)	Main refs.
TP53	Tumor protein p53	10-61%	20, 26, 36, 77-79
CDKN2A	Cyclin-dependent kinase inhibitor 2A (p16INK4)	10-60%	36, 80-82
AXIN1	Axis inhibition protein 1	5-25%	20, 65, 83-87
AXIN2	Axis inhibition protein 2	3-10%	83
HNF1A	Hepatocyte nuclear factor 1a	< 3%	4, 36
RB1	Retinoblastoma 1	< 11%	88
SMAD2-4	SMAD family member 2 and 4	< 10%	89, 90
PTEN	Phosphatase and tensin homolog	< 5-10%	53, 91-94
IGF2R	Insulin-like growth factor 2 receptor	0-13%	95-97
STK11	Serine/threonine-protein kinase 11 (LKB1)	1 mutation	98

Table 3: Oncogenes and tumor suppressor genes mutated in hepatocellular adenomas

Genes altered in adenomas	Protein	% of mutated HCA	Type of mutation	Main refs.
HNF1A	Hepatocyte nuclear factor 1a	35%	Inactivating	4, 6, 35, 99-103
CTNNB1	Catenin (cadherin-associated protein), beta 1 (β -catenin)	15-19%	Activating	29, 34, 35, 104
IL6ST	Interleukin 6 signal transducer (gp130)	35-45%	Activating	5

Figure 1

Significant associations with the transcriptomic classification adapted from Boyault et al, 2007³⁶. The six robust subgroups found in 120 HCC (termed G1 to G6) are shown with their significant relationships with clinical, genetic and oncogenic pathway features.

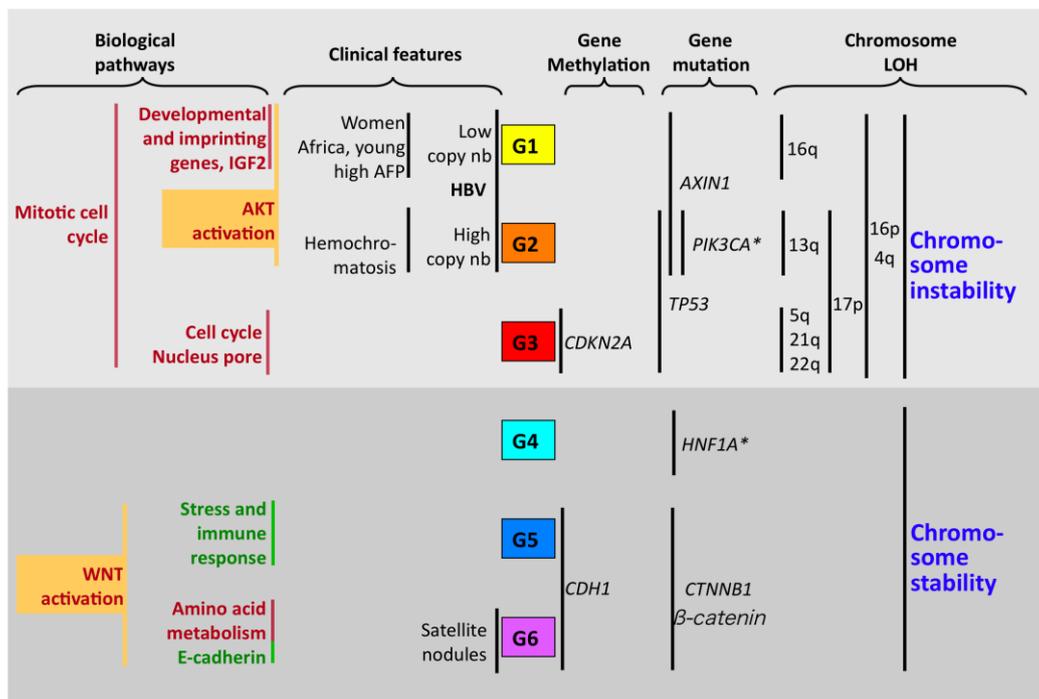
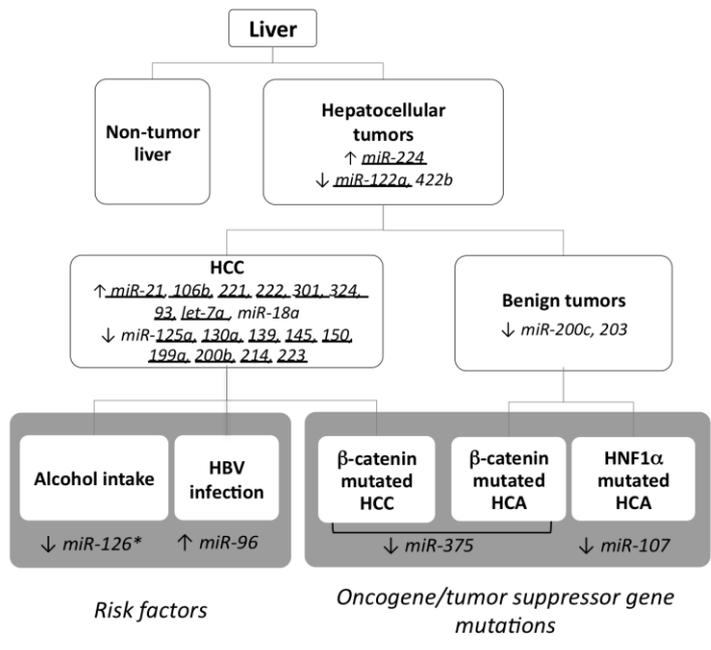


Figure 2

miRNA recurrently altered in HCC and benign liver tumors^{47-51, 53-56}. Major deregulated miRNAs validated in at least two different published studies are indicated.



1. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. *Nat Med* 2004;10:789-99.
2. Laurent-Puig P, Zucman-Rossi J. Genetics of hepatocellular tumors. *Oncogene* 2006;25:3778-86.
3. Forbes SA, Bhamra G, Bamford S, et al. The Catalogue of Somatic Mutations in Cancer (COSMIC). *Curr Protoc Hum Genet* 2008;Chapter 10:Unit 10 1.
4. Bluteau O, Jeannot E, Bioulac-Sage P, et al. Bi-allelic inactivation of TCF1 in hepatic adenomas. *Nat Genet* 2002;32:312-5.
5. Rebouissou S, Amessou M, Couchy G, et al. Frequent in-frame somatic deletions activate gp130 in inflammatory hepatocellular tumours. *Nature* 2009;457:200-4.
6. Bacq Y, Jacquemin E, Balabaud C, et al. Familial liver adenomatosis associated with hepatocyte nuclear factor 1alpha inactivation. *Gastroenterology* 2003;125:1470-5.
7. Lee JS, Chu IS, Heo J, et al. Classification and prediction of survival in hepatocellular carcinoma by gene expression profiling. *Hepatology* 2004;40:667-76.
8. Lee JS, Heo J, Libbrecht L, et al. A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. *Nat Med* 2006;12:410-6.
9. Villanueva A, Newell P, Chiang DY, Friedman SL, Llovet JM. Genomics and signaling pathways in hepatocellular carcinoma. *Semin Liver Dis* 2007;27:55-76.
10. de La Coste A, Romagnolo B, Billuart P, et al. Somatic mutations of the beta-catenin gene are frequent in mouse and human hepatocellular carcinomas. *Proc Natl Acad Sci U S A* 1998;95:8847-51.
11. Miyoshi Y, Iwao K, Nagasawa Y, et al. Activation of the beta-catenin gene in primary hepatocellular carcinomas by somatic alterations involving exon 3. *Cancer Res* 1998;58:2524-7.
12. Nagafuchi A, Takeichi M. Transmembrane control of cadherin-mediated cell adhesion: a 94 kDa protein functionally associated with a specific region of the cytoplasmic domain of E-cadherin. *Cell Regul* 1989;1:37-44.
13. Micsenyi A, Tan X, Sneddon T, Luo JH, Michalopoulos GK, Monga SP. Beta-catenin is temporally regulated during normal liver development. *Gastroenterology* 2004;126:1134-46.
14. Suksaweang S, Lin CM, Jiang TX, Hughes MW, Widelitz RB, Chuong CM. Morphogenesis of chicken liver: identification of localized growth zones and the role of beta-catenin/Wnt in size regulation. *Dev Biol* 2004;266:109-22.
15. Monga SP, Monga HK, Tan X, Mule K, Pediaditakis P, Michalopoulos GK. Beta-catenin antisense studies in embryonic liver cultures: role in proliferation, apoptosis, and lineage specification. *Gastroenterology* 2003;124:202-16.
16. Benhamouche S, Decaens T, Godard C, et al. Apc tumor suppressor gene is the "zonation-keeper" of mouse liver. *Dev Cell* 2006;10:759-70.
17. Cadoret A, Ovejero C, Terris B, et al. New targets of beta-catenin signaling in the liver are involved in the glutamine metabolism. *Oncogene* 2002;21:8293-301.
18. Monga SP, Pediaditakis P, Mule K, Stolz DB, Michalopoulos GK. Changes in WNT/beta-catenin pathway during regulated growth in rat liver regeneration. *Hepatology* 2001;33:1098-109.
19. Tan X, Behari J, Cieply B, Michalopoulos GK, Monga SP. Conditional deletion of beta-catenin reveals its role in liver growth and regeneration. *Gastroenterology* 2006;131:1561-72.

20. Laurent-Puig P, Legoix P, Bluteau O, et al. Genetic alterations associated with hepatocellular carcinomas define distinct pathways of hepatocarcinogenesis. *Gastroenterology* 2001;120:1763-73.
21. Legoix P, Bluteau O, Bayer J, et al. Beta-catenin mutations in hepatocellular carcinoma correlate with a low rate of loss of heterozygosity. *Oncogene* 1999;18:4044-6.
22. Audard V, Grimber G, Elie C, et al. Cholestasis is a marker for hepatocellular carcinomas displaying beta-catenin mutations. *J Pathol* 2007;212:345-52.
23. Cieply B, Zeng G, Proverbs-Singh T, Geller DA, Monga SP. Unique phenotype of hepatocellular cancers with exon-3 mutations in beta-catenin gene. *Hepatology* 2009;49:821-31.
24. Fujito T, Sasaki Y, Iwao K, et al. Prognostic significance of beta-catenin nuclear expression in hepatocellular carcinoma. *Hepatogastroenterology* 2004;51:921-4.
25. Hsu HC, Jeng YM, Mao TL, Chu JS, Lai PL, Peng SY. Beta-catenin mutations are associated with a subset of low-stage hepatocellular carcinoma negative for hepatitis B virus and with favorable prognosis. *Am J Pathol* 2000;157:763-70.
26. Bressac B, Kew M, Wands J, Ozturk M. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa [see comments]. *Nature* 1991;350:429-31.
27. Hsu IC, Metcalf RA, Sun T, Welsh JA, Wang NJ, Harris CC. Mutational hotspot in the p53 gene in human hepatocellular carcinomas. *Nature* 1991;350:427-8.
28. Bluteau O, Beaudoin J-C, Pasturaud P, et al. Specific association between alcohol intake, high grade of differentiation and 4q34-q35 deletions in hepatocellular carcinomas identified by high resolution allelotyping. *Oncogene* 2002;21:1225-32.
29. Chen YW, Jeng YM, Yeh SH, Chen PJ. P53 gene and Wnt signaling in benign neoplasms: beta-catenin mutations in hepatic adenoma but not in focal nodular hyperplasia. *Hepatology* 2002;36:927-35.
30. Laurent-Puig P, Plomteux O, Bluteau O, et al. Frequent mutations of hepatocyte nuclear factor 1 in colorectal cancer with microsatellite instability. *Gastroenterology* 2003;124:1311-4.
31. Rebouissou S, Rosty C, Lecuru F, et al. Mutation of TCF1 encoding hepatocyte nuclear factor 1alpha in gynecological cancer. *Oncogene* 2004;23:7588-92.
32. Rebouissou S, Vasiliu V, Thomas C, et al. Germline hepatocyte nuclear factor 1alpha and 1beta mutations in renal cell carcinomas. *Hum Mol Genet* 2005;14:603-14.
33. Bioulac-Sage P, Laumonier H, Couchy G, et al. Hepatocellular adenoma management and phenotypic classification: The bordeaux experience. *Hepatology* 2009.
34. Van der Borgh S, Libbrecht L, Katoonizadeh A, et al. Nuclear beta-catenin staining and absence of steatosis are indicators of hepatocellular adenomas with an increased risk of malignancy. *Histopathology* 2007;51:855-6.
35. Zucman-Rossi J, Jeannot E, Nhieu JT, et al. Genotype-phenotype correlation in hepatocellular adenoma: new classification and relationship with HCC. *Hepatology* 2006;43:515-24.
36. Boyault S, Rickman DS, de Reynies A, et al. Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. *Hepatology* 2007;45:42-52.
37. Yakicier Y, Legoix P, Vaury C, et al. Identification of homozygous deletions at chromosome 16q23 in Aflatoxin B1 exposed hepatocellular carcinoma. *Oncogene* 2001;20:5232-8.
38. Chen X, Cheung ST, So S, et al. Gene expression patterns in human liver cancers. *Mol Biol Cell* 2002;13:1929-39.
39. Breuhahn K, Vredon S, Haddad R, et al. Molecular profiling of human hepatocellular carcinoma defines mutually exclusive interferon regulation and insulin-like growth factor II overexpression. *Cancer Res* 2004;64:6058-64.

40. Kaposi-Novak P, Lee JS, Gomez-Quiroz L, Coulouarn C, Factor VM, Thorgeirsson SS. Met-regulated expression signature defines a subset of human hepatocellular carcinomas with poor prognosis and aggressive phenotype. *The Journal of clinical investigation* 2006;116:1582-95.
41. Chiang DY, Villanueva A, Hoshida Y, et al. Focal gains of VEGFA and molecular classification of hepatocellular carcinoma. *Cancer Res* 2008;68:6779-88.
42. Coulouarn C, Factor VM, Thorgeirsson SS. Transforming growth factor-beta gene expression signature in mouse hepatocytes predicts clinical outcome in human cancer. *Hepatology* 2008;47:2059-67.
43. Yamashita T, Forgues M, Wang W, et al. EpCAM and alpha-fetoprotein expression defines novel prognostic subtypes of hepatocellular carcinoma. *Cancer Res* 2008;68:1451-61.
44. Hoshida Y, Villanueva A, Kobayashi M, et al. Gene expression in fixed tissues and outcome in hepatocellular carcinoma. *N Engl J Med* 2008;359:1995-2004.
45. Hoshida Y, Nijman SM, Kobayashi M, et al. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. *Cancer Res* 2009;69:7385-92.
46. Cullen BR. Transcription and processing of human microRNA precursors. *Mol Cell* 2004;16:861-5.
47. Ladeiro Y, Couchy G, Balabaud C, et al. MicroRNA profiling in hepatocellular tumors is associated with clinical features and oncogene/tumor suppressor gene mutations. *Hepatology* 2008;47:1955-63.
48. Murakami Y, Yasuda T, Saigo K, et al. Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. *Oncogene* 2006;25:2537-45.
49. Kutay H, Bai S, Datta J, et al. Downregulation of miR-122 in the rodent and human hepatocellular carcinomas. *J Cell Biochem* 2006;99:671-8.
50. Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology* 2007;133:647-58.
51. Gramantieri L, Ferracin M, Fornari F, et al. Cyclin G1 is a target of miR-122a, a microRNA frequently down-regulated in human hepatocellular carcinoma. *Cancer Res* 2007;67:6092-9.
52. Huang YS, Dai Y, Yu XF, et al. Microarray analysis of microRNA expression in hepatocellular carcinoma and non-tumorous tissues without viral hepatitis. *J Gastroenterol Hepatol* 2008;23:87-94.
53. Wang Y, Lee AT, Ma JZ, et al. Profiling microRNA expression in hepatocellular carcinoma reveals microRNA-224 up-regulation and apoptosis inhibitor-5 as a microRNA-224-specific target. *J Biol Chem* 2008;283:13205-15.
54. Jiang J, Gusev Y, Aderca I, et al. Association of MicroRNA expression in hepatocellular carcinomas with hepatitis infection, cirrhosis, and patient survival. *Clin Cancer Res* 2008;14:419-27.
55. Varnholt H, Drebber U, Schulze F, et al. MicroRNA gene expression profile of hepatitis C virus-associated hepatocellular carcinoma. *Hepatology* 2008;47:1223-32.
56. Connolly E, Melegari M, Landgraf P, et al. Elevated expression of the miR-17-92 polycistron and miR-21 in hepadnavirus-associated hepatocellular carcinoma contributes to the malignant phenotype. *Am J Pathol* 2008;173:856-64.
57. Budhu A, Jia HL, Forgues M, et al. Identification of metastasis-related microRNAs in hepatocellular carcinoma. *Hepatology* 2008;47:897-907.
58. Ji J, Shi J, Budhu A, et al. MicroRNA expression, survival, and response to interferon in liver cancer. *N Engl J Med* 2009;361:1437-47.

59. Ji J, Yamashita T, Budhu A, et al. Identification of microRNA-181 by genome-wide screening as a critical player in EpCAM-positive hepatic cancer stem cells. *Hepatology* 2009;50:472-80.
60. Ura S, Honda M, Yamashita T, et al. Differential microRNA expression between hepatitis B and hepatitis C leading disease progression to hepatocellular carcinoma. *Hepatology* 2009;49:1098-112.
61. Huang XH, Wang Q, Chen JS, et al. Bead-based microarray analysis of microRNA expression in hepatocellular carcinoma: miR-338 is downregulated. *Hepatol Res* 2009;39:786-94.
62. Su H, Yang JR, Xu T, et al. MicroRNA-101, down-regulated in hepatocellular carcinoma, promotes apoptosis and suppresses tumorigenicity. *Cancer Res* 2009;69:1135-42.
63. Coulouarn C, Factor VM, Andersen JB, Durkin ME, Thorgeirsson SS. Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. *Oncogene* 2009;28:3526-36.
64. Huang H, Fujii H, Sankila A, et al. Beta-catenin mutations are frequent in human hepatocellular carcinomas associated with hepatitis C virus infection. *Am J Pathol* 1999;155:1795-801.
65. Taniguchi K, Roberts LR, Aderca IN, et al. Mutational spectrum of beta-catenin, AXIN1, and AXIN2 in hepatocellular carcinomas and hepatoblastomas. *Oncogene* 2002;21:4863-71.
66. Terris B, Pineau P, Bregeaud L, et al. Close correlation between beta-catenin gene alterations and nuclear accumulation of the protein in human hepatocellular carcinomas. *Oncogene* 1999;18:6583-8.
67. Wong CM, Fan ST, Ng IO. beta-Catenin mutation and overexpression in hepatocellular carcinoma: clinicopathologic and prognostic significance. *Cancer* 2001;92:136-45.
68. Challen C, Guo K, Collier JD, Cavanagh D, Bassendine MF. Infrequent point mutations in codons 12 and 61 of ras oncogenes in human hepatocellular carcinomas. *J Hepatol* 1992;14:342-6.
69. Leon M, Kew MC. Analysis of ras gene mutations in hepatocellular carcinoma in southern African blacks. *Anticancer Res* 1995;15:859-61.
70. Takada S, Koike K. Activated N-ras gene was found in human hepatoma tissue but only in a small fraction of the tumor cells. *Oncogene* 1989;4:189-93.
71. Tsuda H, Hirohashi S, Shimosato Y, Ino Y, Yoshida T, Terada M. Low incidence of point mutation of c-Ki-ras and N-ras oncogenes in human hepatocellular carcinoma. *Jpn J Cancer Res* 1989;80:196-9.
72. Weihrauch M, Benicke M, Lehnert G, Wittekind C, Wrbitzky R, Tannapfel A. Frequent k-ras -2 mutations and p16(INK4A) methylation in hepatocellular carcinomas in workers exposed to vinyl chloride. *Br J Cancer* 2001;84:982-9.
73. Lee JW, Soung YH, Kim SY, et al. PIK3CA gene is frequently mutated in breast carcinomas and hepatocellular carcinomas. *Oncogene* 2005;24:1477-80.
74. Tanaka Y, Kanai F, Tada M, et al. Absence of PIK3CA hotspot mutations in hepatocellular carcinoma in Japanese patients. *Oncogene* 2006;25:2950-2.
75. Park WS, Dong SM, Kim SY, et al. Somatic mutations in the kinase domain of the Met/hepatocyte growth factor receptor gene in childhood hepatocellular carcinomas. *Cancer Res* 1999;59:307-10.
76. Yang DH, Huang W, Cui J, et al. The relationship between point mutation and abnormal expression of c-fms oncogene in hepatocellular carcinoma. *Hepatobiliary Pancreat Dis Int* 2004;3:86-9.

77. Hayashi H, Sugio K, Matsumata T, Adachi E, Takenaka K, Sugimachi K. The clinical significance of p53 gene mutation in hepatocellular carcinomas from Japan. *Hepatology* 1995;22:1702-7.
78. Honda K, Sbisa E, Tullo A, et al. p53 mutation is a poor prognostic indicator for survival in patients with hepatocellular carcinoma undergoing surgical tumour ablation. *Br J Cancer* 1998;77:776-82.
79. Hussain SP, Schwank J, Staib F, Wang XW, Harris CC. TP53 mutations and hepatocellular carcinoma: insights into the etiology and pathogenesis of liver cancer. *Oncogene* 2007;26:2166-76.
80. Chen TC, Hsieh LL, Kuo TT, et al. p16INK4 gene mutation and allelic loss of chromosome 9p21-22 in Taiwanese hepatocellular carcinoma. *Anticancer Res* 2000;20:1621-6.
81. Kita R, Nishida N, Fukuda Y, et al. Infrequent alterations of the p16INK4A gene in liver cancer. *Int J Cancer* 1996;67:176-80.
82. Liew CT, Li HM, Lo KW, et al. High frequency of p16INK4A gene alterations in hepatocellular carcinoma. *Oncogene* 1999;18:789-95.
83. Ishizaki Y, Ikeda S, Fujimori M, et al. Immunohistochemical analysis and mutational analyses of beta-catenin, Axin family and APC genes in hepatocellular carcinomas. *Int J Oncol* 2004;24:1077-83.
84. Kim YD, Park CH, Kim HS, et al. Genetic alterations of Wnt signaling pathway-associated genes in hepatocellular carcinoma. *J Gastroenterol Hepatol* 2008;23:110-8.
85. Park JY, Park WS, Nam SW, et al. Mutations of beta-catenin and AXIN I genes are a late event in human hepatocellular carcinogenesis. *Liver Int* 2005;25:70-6.
86. Satoh S, Daigo Y, Furukawa Y, et al. AXIN1 mutations in hepatocellular carcinomas, and growth suppression in cancer cells by virus-mediated transfer of AXIN1. *Nat Genet* 2000;24:245-50.
87. Zucman-Rossi J, Benhamouche S, Godard C, et al. Differential effects of inactivated Axin1 and activated beta-catenin mutations in human hepatocellular carcinomas. *Oncogene* 2007;26:774-80.
88. Zhang X, Xu HJ, Murakami Y, et al. Deletions of chromosome 13q, mutations in Retinoblastoma 1, and retinoblastoma protein state in human hepatocellular carcinoma. *Cancer Res* 1994;54:4177-82.
89. Kawate S, Takenoshita S, Ohwada S, et al. Mutation analysis of transforming growth factor beta type II receptor, Smad2, and Smad4 in hepatocellular carcinoma. *Int J Oncol* 1999;14:127-31.
90. Yakicier MC, Irmak MB, Romano A, Kew M, Ozturk M. Smad2 and Smad4 gene mutations in hepatocellular carcinoma. *Oncogene* 1999;18:4879-83.
91. Bae JJ, Rho JW, Lee TJ, et al. Loss of heterozygosity on chromosome 10q23 and mutation of the phosphatase and tensin homolog deleted from chromosome 10 tumor suppressor gene in Korean hepatocellular carcinoma patients. *Oncol Rep* 2007;18:1007-13.
92. Fujiwara Y, Hoon DS, Yamada T, et al. PTEN / MMAC1 mutation and frequent loss of heterozygosity identified in chromosome 10q in a subset of hepatocellular carcinomas. *Jpn J Cancer Res* 2000;91:287-92.
93. Kawamura N, Nagai H, Bando K, et al. PTEN/MMAC1 mutations in hepatocellular carcinomas: somatic inactivation of both alleles in tumors. *Jpn J Cancer Res* 1999;90:413-8.
94. Yao YJ, Ping XL, Zhang H, et al. PTEN/MMAC1 mutations in hepatocellular carcinomas. *Oncogene* 1999;18:3181-5.
95. De Souza AT, Hankins GR, Washington MK, Orton TC, Jirtle RL. M6P/IGF2R gene is mutated in human hepatocellular carcinomas with loss of heterozygosity. *Nat Genet* 1995;11:447-9.

96. Oka Y, Waterland RA, Killian JK, et al. M6P/IGF2R tumor suppressor gene mutated in hepatocellular carcinomas in Japan. *Hepatology* 2002;35:1153-63.
97. Yamada T, De Souza AT, Finkelstein S, Jirtle RL. Loss of the gene encoding mannose 6-phosphate/insulin-like growth factor II receptor is an early event in liver carcinogenesis. *Proc Natl Acad Sci U S A* 1997;94:10351-5.
98. Kim CJ, Cho YG, Park JY, et al. Genetic analysis of the LKB1/STK11 gene in hepatocellular carcinomas. *Eur J Cancer* 2004;40:136-41.
99. Jeannot E, Poussin K, Chiche L, et al. Association of CYP1B1 germ line mutations with hepatocyte nuclear factor 1alpha-mutated hepatocellular adenoma. *Cancer Res* 2007;67:2611-6.
100. Jeannot E, Wendum D, Paye F, et al. Hepatocellular adenoma displaying a HNF1alpha inactivation in a patient with familial adenomatous polyposis coli. *J Hepatol* 2006;45:883-6.
101. Pelletier L, Rebouissou S, Paris A, et al. Loss of hepatocyte nuclear factor 1alpha function in human hepatocellular adenomas leads to aberrant activation of signaling pathways involved in tumorigenesis. *Hepatology* 2010;51:557-66.
102. Rebouissou S, Imbeaud S, Balabaud C, et al. HNF1alpha inactivation promotes lipogenesis in human hepatocellular adenoma independently of SREBP-1 and carbohydrate-response element-binding protein (ChREBP) activation. *J Biol Chem* 2007;282:14437-46.
103. Reznik Y, Dao T, Coutant R, et al. Hepatocyte nuclear factor-1 alpha gene inactivation: cosegregation between liver adenomatosis and diabetes phenotypes in two maturity-onset diabetes of the young (MODY)3 families. *J Clin Endocrinol Metab* 2004;89:1476-80.
104. Bioulac-Sage P, Rebouissou S, Thomas C, et al. Hepatocellular adenoma subtype classification using molecular markers and immunohistochemistry. *Hepatology* 2007;46:740-8.