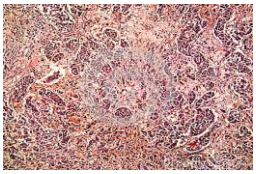
Молекулярно-генетические исследования при первичном раке печени

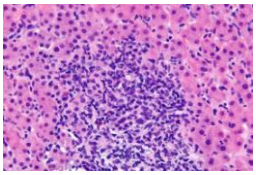
д.м.н. Любченко Л.Н.
ФГБУ «РОНЦ им. Н.Н. Блохина» МЗ РФ

Первичный рак печени

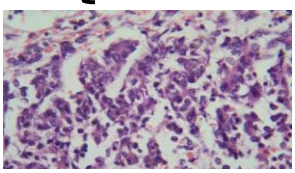
1. **гепатоцеллюлярный рак 85-90%**

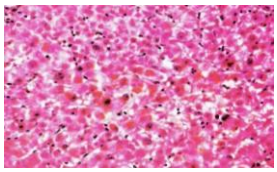


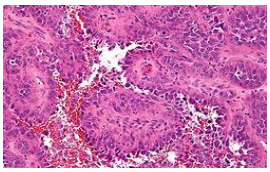
2. **холангиокарцинома – 5-10%**



<5%
3. **гепатобластома**
4. **фиброламеллярный подтип ГЦР**
5. **ангиосаркома**

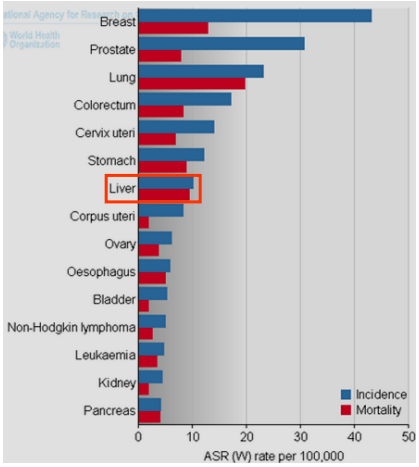






International Agency for Research on Cancer
World Health Organization

GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012

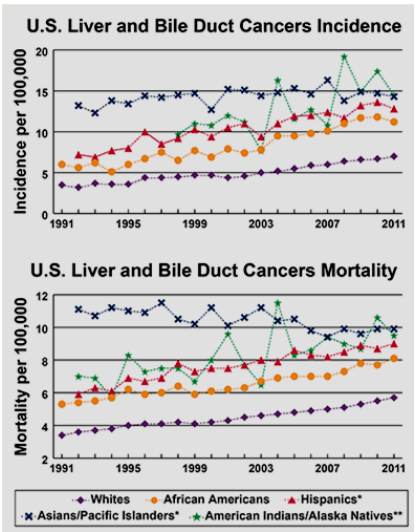


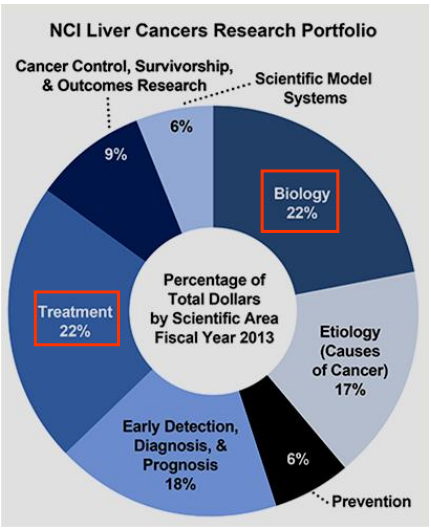
<u>Мультифакториальная природа</u>:

-**HBV, HCV**
-**Токсическое поражение**
-**Метаболический синдром**
-**Билиарный цирроз**
-**Гемохроматоз**
-**Афлатоксин В1**

- **Самая частая первичная опухоль печени**
- **6-7 место в структуре заболеваемости**
- **3 место в структуре смертности**

NIH NATIONAL CANCER INSTITUTE





Рост заболеваемости в связи с ростом HCV, HBV

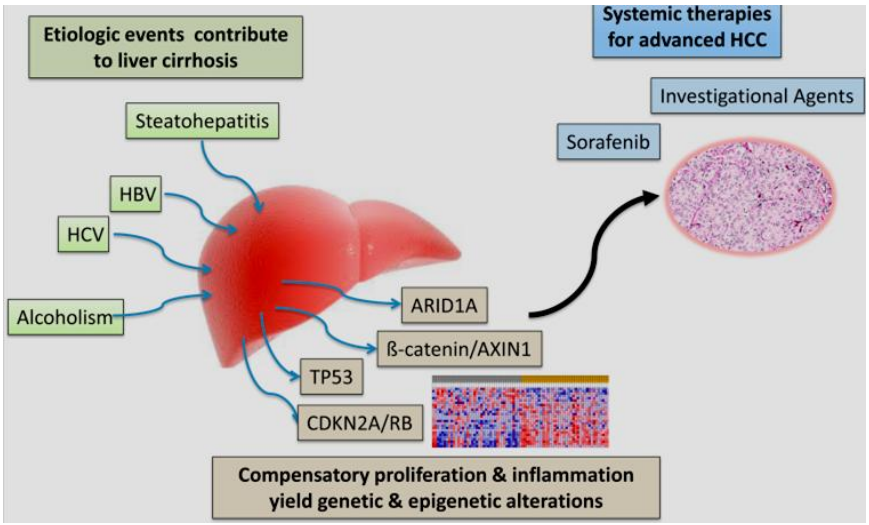
Персонализированная медицина - приоритетное направление

ONCRU16NP00942

The Changing Landscape of Hepatocellular Carcinoma

Etiology, Genetics, and Therapy

Erik S. Knudsen,*† Purva Gopal,* and Amit G. Singal†‡



CASE REPORT Open Access

Familial hepatocellular carcinoma in an endemic area: two case reports

Weledji et al. BMC Res Notes (2015) 8:415
DOI 10.1186/s13104-015-1366-7

Elroy P. Weledji[1*], Dickson S. Nsagha[2], George Enoworock[3] and Maurice Mouladje[3]

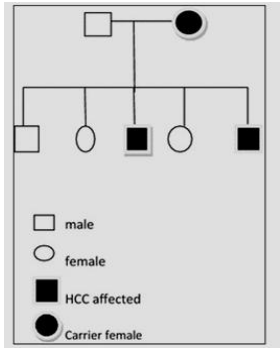


Table 1 Familial hepatocellular carcinoma

1965	Kaplan and Cole [20]	3 male adult siblings aged 64, 49 and 44
1968	Hagstrom and Baker [21]	3 male siblings aged 11, 22 and 31
1971	Denison et al. [22]	Familial hepatoma on background micro-nodular cirrhosis
1972	Ohbayashi et al. [17]	Familial clustering of asymptomatic carriers
1981	Gilmore et al. [29]	3 or 4 male siblings in Chinese family
1984	Lynch et al. [7]	2 familial aggregations in Costa Rica
1984	Harvey et al. [23]	3 male siblings aged 33, 43, 46
1984	Chang et al. [24]	2 pairs of young brothers (5 and 7years) and (9 and 7years)
1991	Lok et al. [25]	Morbidity and mortality from chronic hepatitis B virus infection in family members
1991	Alberts et al. [26]	Clustering of hepatocellular carcinoma in Alaska Native families
2014	Weledji et al. [3] *BMC Research notes*	2 male siblings aged, 24 and 35 in endemic area in Cameroon

Table 3: Reports of FLC in the setting of inherited syndromes or with other nonhepatic tumors.

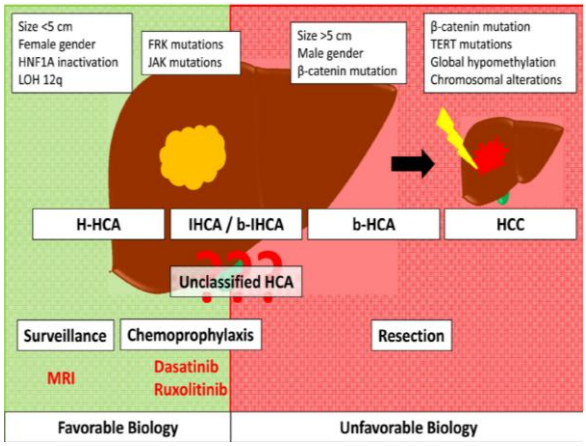
Syndrome
Wilms [51]
Carney [74]
Fanconi anemia [110]
FAP [111]

Молекулярная классификация гепатоцеллюлярных аденом

Cancer Cell. 2014 April 14; 25(4): 409–411. doi:10.1016/j.ccr.2014.03.032.

Next-generation Genomic Profiling of Hepatocellular Adenomas: A New Era of Individualized Patient Care

Jens U. Marquardt[1,2] and **Snorri S. Thorgeirsson**[1]



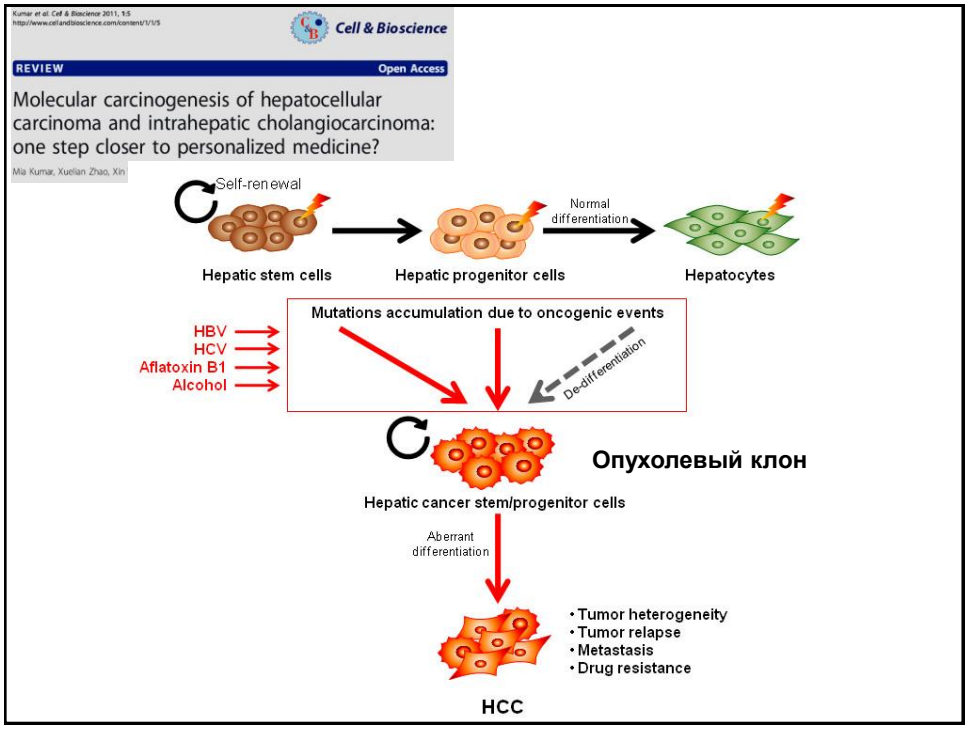
- Гормонально-чувствительные
- Чаще у женщин

- **H-HCA подтип**:
-mutHNF1α в 30-40%
-риск сахарного диабета

- **I-HCA подтип**:
mutIL6ST, *GNAS*, *STAT3*
mutβ-catenin до 50%

- **b-HCA подтип**

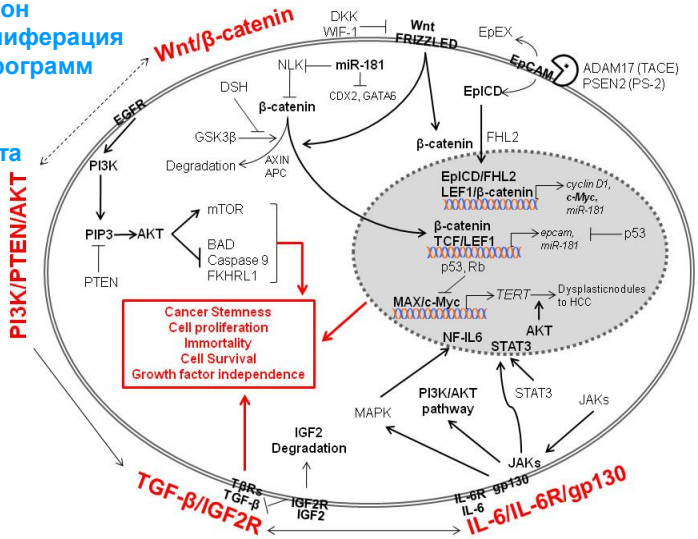
mutβ-catenin:
высокий потенциал *озлокачествления*



Сигнальные пути, вовлеченные в канцерогенез ГЦР

130 сигнальных путей активировано при ГЦР

- **Опухолевый клон**
- **Клеточная пролиферация**
- **Выключение программ апоптоза**
- **Независимость от факторов роста**



Cell & Bioscience2011

Mini-review

Next generation sequencing reveals genetic landscape of hepatocellular carcinomas

Shuyu Li [a,*], Mao Mao [b,*]

[a] LRL IT China, Shanghai, China

S. Li, M. Mao / Cancer Letters xxx (2012) xxx–xxx

Полноэкзомное, -геномное секвенирование при ГЦР

Table 1
Published NGS studies on liver cancers.

Ref.	Study design	Summary of results
[32] 2011	WGS and WES of a HCV positive HCC	Identified and validated 63 non-synonymous somatic substitutions including mutations of TP53 and AXIN1. Identified and validated 22 chromosome rearrangements generating four fusion transcripts
[55] 2011	WGS or WES of nine tumor samples and seven adjacent normal liver samples from a HBV positive HCC	Identified and validated 24 somatic mutations that alter amino acid sequences. Evolution of tumors was inferred from mutations derived from multiple primary and recurrent tumor samples
[37] 2011	WES of 10 HCV positive HCCs. Screening of five recurrently mutated genes in an additional cohort of 129 HCCs associated various disease etiologies	Identified frequent mutations in CTNNB1 and TP53. Identified novel and frequent inactivating mutations in ARID2 gene. Mutation frequencies of CTNNB1, TP53 and ARID2 are associated with disease etiology
[31] 2012	WGS of four HCCs (3 HBV positive and 1 HBV negative)	Identified 255 HBV integration sites. HBV integration into MLL4, ANGPT1, and a novel transcript resulted in elevated gene expression
[30] 2012	WES of 24 HCCs, among them one is HBV positive, four are HCV positive, 12 are alcohol related. Screening of 14 genes in an additional cohort of 125 HCCs	Identified frequent mutations in CTNNB1 and TP53. Identified novel and frequent inactivating mutations in ARID1A, ARID2 and other genes involved in chromatin remodeling. Mutation frequencies of these genes are associated with disease etiology
[29] 2012	WGS of 27 HCCs (11 HBV positive, 15 HCV positive, two non-viral). Screening of significantly mutated genes in an additional cohort of 120 HCCs	Identified frequent and novel mutations in genes involved in chromatin remodeling, e.g. ARID1A, ARID1B, ARID2, MLL and MLL3. HBV integration in TERT gene in 4 of the 11 HBV positive HCCs
[49] 2012	WGS of 88 HCCs (81 HBV positive, 7 HBV negative)	Recurrent HBV integration in TERT, MLL4 and CCNE1. HBV integrations led to elevated gene expression. HBV integration associated with chromosome instability, early onset and poor outcome
[33] 2012	WES of 10 HBV positive HCCs. Screening 10 significantly mutated genes in an additional cohort of 100 HCCs	ARID1A mutations in 13% of HBV associated HCCs. Functional study suggested ARID1A mutation may be crucial in HCC invasion and metastasis
[40] 2012	WES of eight cholangiocarcinomas (CCAs). Screening of 15 significantly mutated in 46 additional CCAs	Identified frequent mutations in TP53, KRAS, SMAD4 and MLL3

International Hepatology

CrossMark

Journal of Hepatology 2014 vol. 60 | 224–226

Genetics of hepatocellular carcinoma

Jean-Charles Nault[1,2,3,4,*], Jessica Zuc

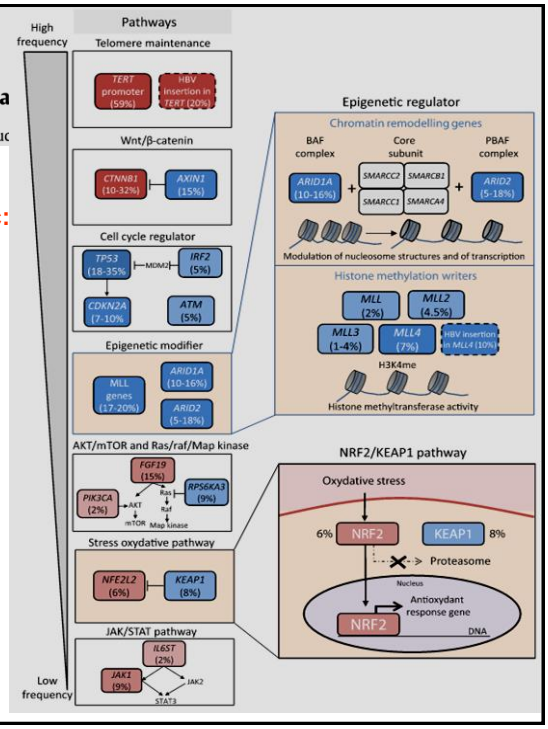
Активация Wnt/CTNNB1-пути – основной этап в онкогенезе ГЦР с:

1. ***mtTERT до 60%***
2. ***mtCTNNB1 до 40%***
3. ***mtAXIN1 до15%***

Полноэкзомный сиквенс

- **87 ГЦК (норма/опухоль)**
- **45 несинонимичные mt**

- ***mtTP53* - 18%**
- ***mtCTNNB1* - 10%**
- ***mtKEAP1* – 8%**
- ***mtC16orf62* – 8%**
- ***mtMLL4* – 7%**
- ***mtRAC2* – 5%**



Research
23:1422–1433 © 2013, Published by Cold Spring Harbor Laboratory Press; ISSN 1088-9051/13; www.genome.org

Whole-genome sequencing identifies recurrent mutations in hepatocellular carcinoma

Zhengyan Kan,[1,13] Hancheng Zheng,[2,13] Xiao Liu,[2,3,13] Shuyu Li,[4,13] Thomas D. Barber,[4]

N=88 ГЦР (норма/опухоль)

Table 1. Significantly mutated genes in primary HCC

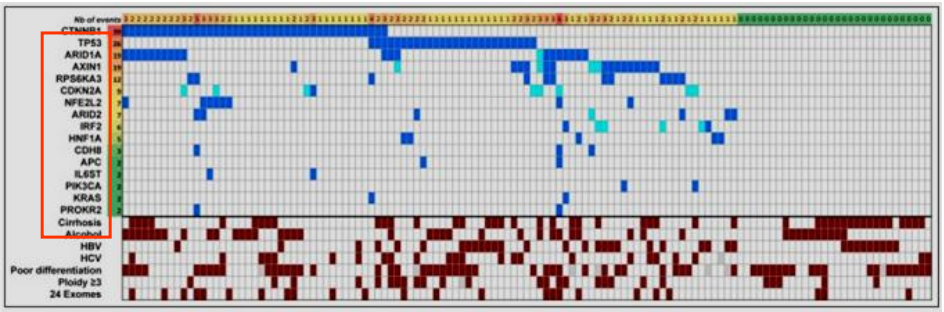
Gene	Description	Mutation frequency	Confidence interval (95%)	No. COSMIC matched
TP53	Tumor protein p53	35.2% (31)	±10.0%	29
CTNNB1	Catenin (cadherin-associated protein), beta 1, 88 kDa	15.9% (14)	±7.6%	12
JAK1	Janus kinase 1	9.1% (8)	±6.0%	2
AXIN1	Axin 1	4.5% (4)	±4.4%	0
EPS15	Epidermal growth factor receptor pathway substrate 15	4.5% (4)	±4.4%	0
SLC10A1	Solute carrier family 10 (sodium/bile acid cotransporter family), member 1	3.4% (3)	±3.6%	0
CACNA2D4	Calcium channel, voltage-dependent, alpha2/delta subunit 4	5.7% (5)	±4.8%	0
ADCY2	Adenylate cyclase 2 (brain)	5.7% (5)	±4.8%	0
LRP1B	Low-density lipoprotein receptor-related protein 1B	11.4% (10)	±6.6%	0
FAM5C	Family with sequence similarity 5, member C	5.7% (5)	±4.8%	0
COL11A1	Collagen, type XI, alpha 1	6.8% (6)	±5.3%	0

Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma

Nat Genet. 2012 Jun; 44(6): 694–698.

Cécile Guichard, Giuliana Amaddeo, [...], and Jessica Zucman-Rossi

N=125 ГЦР
N=24 (whole-exome sequencing)
994 соматических мутаций
135 гомозиготных делеций



- новые гены *ARID1A, RPS6KA3, NFE2L2, IRF2, CDH8 и PROKR2*
- ***mtCTNNB1 (32,5%), mtTP53 (20,8%), mtARID1A (16,8%)***
- ***mtCTNNB1, mtAXIN1, mtAPC*** **взаимо-исключают друг друга**
- ***mtCTNNB1*** **– HBV-негативный ГЦР**
- ***mtAXIN1 и mtAPC*** **– HBV-позитивный ГЦР**
- **ГЦР с высоким числом Indels:**

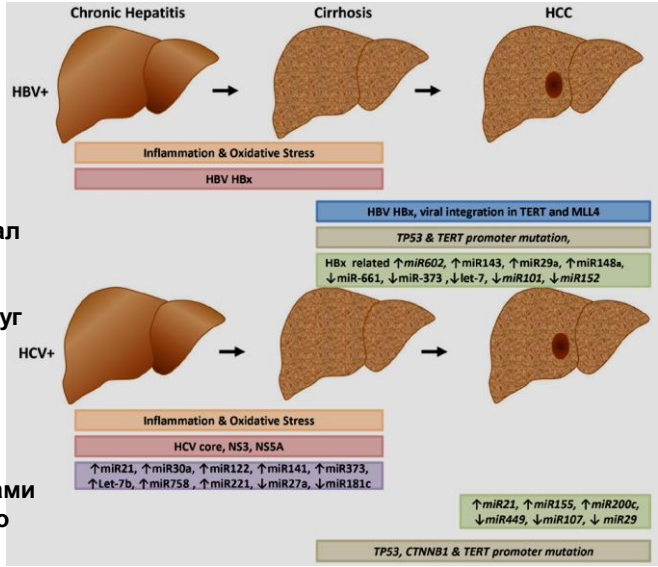
отсутствие цирроза, HBV+, большие размеры, низкая ст. дифференцировки, высокий АФП, агрессивное течение

www.impactjournals.com/oncotarget/ Oncotarget, Vol. 7, No. 1

Molecular alterations in hepatocellular carcinoma associated with hepatitis B and hepatitis C infections

Maria Lina Tornesello[1,*], Luigi Buonaguro[1,*], Francesco Izzo[2], Franco M. Buonaguro[1]

- ***mtTP53*** **определяют агрессивное течение**
- ***mtCTNNB1*** **и** ***mtTP53*** **определяют потенциал злокачественной трансформации взаимоисключают друг друга**
- ***mtTERT*** **в 25% диспластических аденомах печени и являются предикторами высокого онкогенного потенциала**

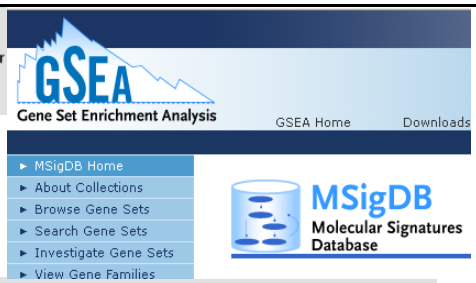


Cancer Res 2009 September 15; 69(18): 7385–7392. doi:10.1158/0008-5472.CAN-09-1089.

Integrative Transcriptome Analysis Reveals Common Molecular Subclasses of Human Hepatocellular Carcinoma

Yujin Hoshida[1,2], Sebastian M.B. Nijman[1,3], Masahiro Kobayashi[4], Jennifer A. Chan[1,5]

- **N=603 пациента**
- **Восточная и западная Европа**
- **619 генов**



Clinical phenotypes associated with HCC subclasses

Variable		S1	S2	S3	p-value
Tumor size (cm)*		3.0 [2.0,4.5]	4.5 [2.5,7.0]	2.5 [1.8,4.3]	0.003
Tumor differentiation*	Well	8 (16%)	4 (10%)	37 (44%)	
	Moderate	27 (53%)	23 (59%)	45 (53%)	<0.001
	Poor	16 (31%)	12 (31%)	3 (4%)	
alpha-fetoprotein (ng/ml)†		50 [14,332]	171 [27,1251]	13 [5,43]	<0.001
Hepatitis B virus infection‡		39 (38%)	27 (36%)	39 (25%)	0.05
Hepatitis C virus infection‡		55 (53%)	44 (58%)	109 (69%)	0.03

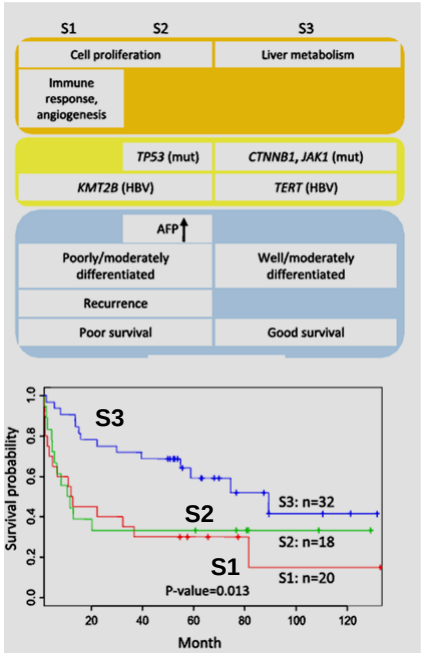
Молекулярная Классификация ГЦР

S1-S2
- Низкая ст. дифференцировки
- Высокий риск рецидивов
- HBV

S2
- *mtTP53*
- Высокий АФП

S3
- *mtCTNNB1, mtJAK1*
- *mtTERT* (HBV)

Kan et al., 2013



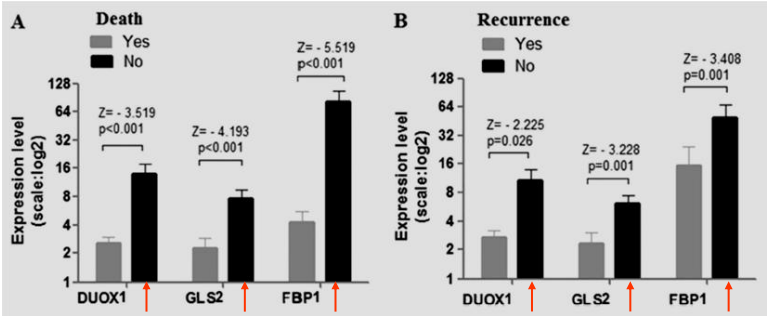
The combination of three molecular markers can be a valuable predictive tool for the prognosis of hepatocellular carcinoma patients

Sheng-Sen Chen, Kang-Kang Yu, Qing-Xia Ling, Chong Huang, Ning Li, Jian-Ming Zheng,

2016

- N=72 ГЦР (норма/опухоль)
- *DUOX1*
- *GLS2*
- *FBP1*

→ Маркеры супрессии опухолевого роста



Статистически достоверное снижение показателей смертности и рецидива ГЦР при повышенной экспрессии *DUOX1, GLS2, FBP1*

		Predicted prognosis		
Actual prognosis	Group size	Non death	Death	Correct percentage
Non death	27	24	3	88.9%
Death	45	2	43	95.6%
Overall percent				93.1%
		Recurrence	Non recurrence	
Recurrence	35	29	6	82.9%
Non recurrence	37	4	33	89.2%
Overall percent				86.1%

Gastroenterology. 2013 May ; 144(5): 1066–1075.e1. doi:10.1053/j.gastro.2013.01.054.

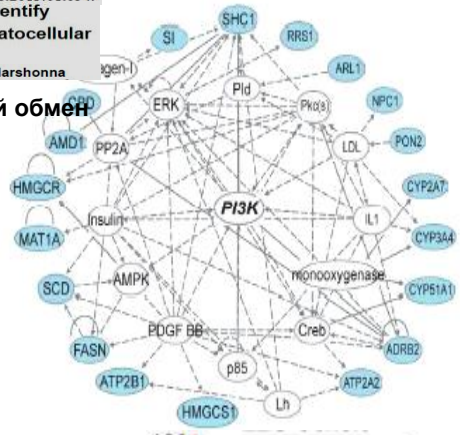
Integrated Metabolite and Gene Expression Profiles Identify Lipid Biomarkers Associated with Progression of Hepatocellular Carcinoma and Patient Outcomes

Anuradha Budhu[1], Stephanie Roessler[1], Xuelian Zhao[1], Zhipeng Yu[1], Marshonna

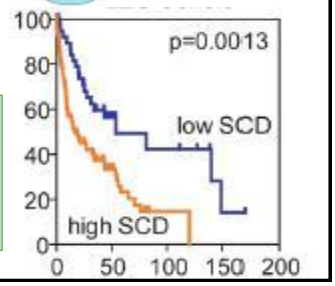
Анализ генов, вовлеченных в липидный обмен при ГЦР

247 пациентов с ГЦР

образцы опухолевой и нормальной ткани
EpCAM+ AFP+ низко-дифференцированная ГЦК
и EpCAM- AFP- высоко-дифференцированная ГЦК



выявлено 28 биохимических маркеров (метаболиты жирных кислот) и 169 генов, повышение экспрессии которых, ассоциировано с агрессивным течением ГЦР, худшей ОВ и БРВ



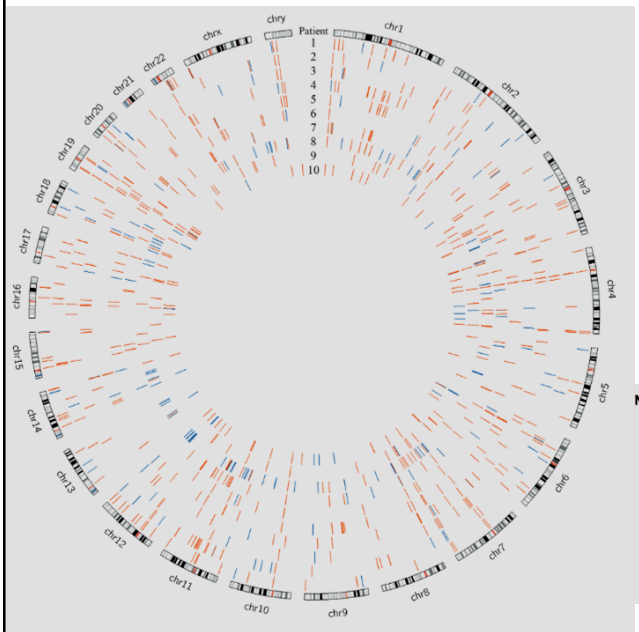
Hepatocellular Carcinoma, Fibrolamellar Variant: Diagnostic Pathologic Criteria and Molecular Pathology Update. A Primer

Consolato M. Sergi

Diagnostics (Basel). 2016 Mar; 6(1): 3.

Фиброламеллярный вариант ГЦР

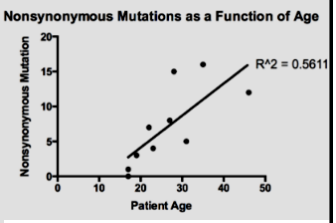
- **Молодой возраст**
- **Преобладают женщины**
- **Отсутствие HBV**
- **Отсутствие цирроза печени**
- **Менее агрессивное течение**
- **Нормальный уровень АФП**
- **Del 19 хромосомы**
- **Химерный транскрипт *DNAJB1/PRKACA***



gure 2: Genomic deletions in HCC (blue) and FL-HCC (orange) across ten patients each, demonstrating distinct itational spectra.

Классический вариант ГЦР

Фиброламелярный вариант ГЦР



Diagnostics (Basel). 2016 Mar; 6(1): 3.

Personalized therapy for hepatocellular carcinoma: Where are we now?

Stephen L. Chan [a,b,c,*], Alissa M. Wong [d], Kirsty Lee [b], Nathalie Wong [d], Allen K.C. Chan [a,e]

[a] State Key Laboratory of Oncology in South China, The Chinese University of Hong Kong, Hong Kong

Cancer Treatment Reviews 45 (2016) 77–86

Таргетные препараты в лечении ГЦР

Summary of targeted agents with evidence of biomarkers for HCC.

Drug	Predictive biomarker	Biomarker-enriched clinical trial	Estimated proportion of biomarker positivity in the HCC population
Ramucirumab	High serum AFP level (⩾400 ng/mL)	REACH2 (phase III; NCT02435433)	30–40%
c-MET inhibitor	c-MET-high (IHC) MET copy number (FISH)	Tivantinib (phase III; NCT01755767) INC280 (phase I/II; NCT01737827) MSC2156119 J (phase I; NCT01988493)	5–40% (dependent on the definition of criteria)
Everolimus	TSC2 loss	Under planning	10–20%
HDAC inhibitor	HR23B HDAC mutations	Under planning	20–30%
FGFR4 inhibitor	Expression of FGFR4/FGF19/beta-Klotho	FGF401 (phase I; NCT02325739) BLU554 (phase I; NCT02508467)	5–10%

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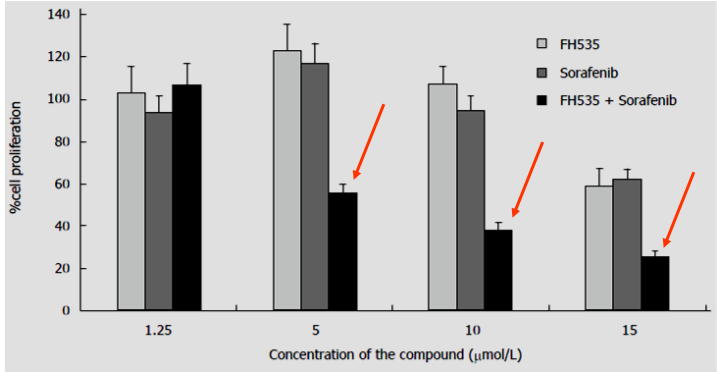
REVIEW

Targeting Wnt/β-catenin pathway in hepatocellular carcinoma treatment

Valery Vilchez, Lilia Turcios, Francesc Marti, Roberto Gedaly



- **Сорафениб – стандарт в лечении пациентов с диссем. ГЦР**
- **Синергичный эффект при одновременном использовании ингибиторов:**

-***RAS/RAS/MAPK* –пути**
-***PI3K/AKT/mTOR* – пути**
-***Wnt/CTNNB1* – пути -** **Снижение клеточной пролиферации**

Personalized therapy for hepatocellular carcinoma: Where are we now?

Stephen L. Chan [a,b,c,*], Alissa M. Wong [d], Kirsty Lee [b], Nathalie Wong [d], Allen K.C. Chan [a,e]
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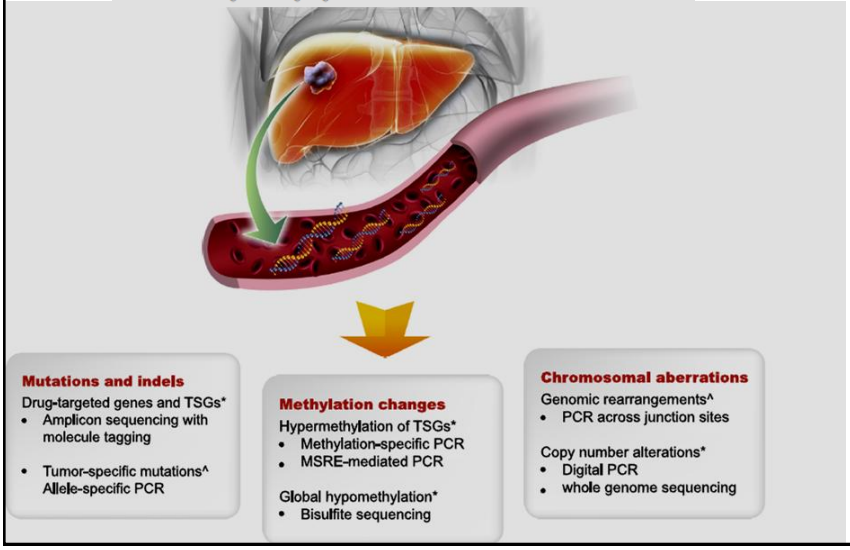
Cancer Treatment Reviews 45 (2016) 77–86

Liquid Biopsy and its Potential for Management of Hepatocellular Carcinoma

J Gastrointest Canc
DOI 10.1007/s12029-016-9801-0

Jian Zhou[1] · Ao Huang[1] · Xin-Rong Yang[1]



Liquid Biopsy in Liver Cancer

Published on April 29, 2015

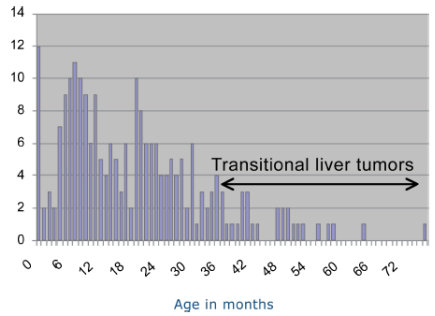
Author: Ismail Labgaa

Table 1. Summary of the Role of "Liquid Biopsy" Elements, as Diagnostic and Prognostic Tools in Clinical Setting.

"Liquid biopsy" elements	Role	Number of HCC patients	BCLC Stage 0+A	BCLC Stage B+C	Biomarkers	Technic used for detection	Outcomes	References
CTC	Diagnosis	85	NA		CTC detection	Antibody-coated magnetic beads		Xu *et al.*, 2011
	Prognosis				Number of CTC per 5ml		Tumor size, portal vein thrombus, differentiation status, TNM stage and Milan criteria	
		82	NA		Circulating cancer stem cells (CSC)	Multicolor flow cytometry	Intra- and extra-hepatic recurrence, survival after liver resection	Fan *et al.*, 2011
		44				Isolation by size of epithelial tumor cells (ISET)	Tumor invasion, portal vein thrombus and survival	Vona *et al.*, 2004
		123	101	22	EpCAM+	EpCAM antibody-Coated magnetic beads (CellSearch)	Recurrence after liver resection	Sun *et al.*, 2013
		60	NA	NA	ICAM-1	Flow cytometry	Survival	Liu *et al.*, 2013
		299	119*	38*	EpCAM+	Coated magnetic beads (CellSearch)	Response to treatment	Guo *et al.*, 2014
		96	53	43	Circulating cancer stem cells (CSC)	Magnetic cell sorting (MCS)	Tumor grade, size, BCLC stage and recurrence	Cheng *et al.* 2013
ctDNA	Diagnosis	50	NA		Epigenetic changes of RASSF1A, p16, and p15	Methylation-specific PCR		Zhang *et al.* 2007
		72	NA		Plasma methylation analysis of a panel of four genes (APC, GSTP1, ASSF1A, and SFRP1)	Methylation-sensitive restriction enzymes-based quantitative PCR		Huang *et al.*, 2011
	Prognosis				RASSF1A methylation		Overall survival	
cfRNA	Diagnosis	22	NA		miR-92a	Quantitative RT-PCR		Shigoka *et al.*, 2010
	Prognosis	NA	NA		Vps4A		TNM stage, tumor size, tumor capsule integrity, recurrence free-survival	Wei *et al.*, 2014

Гепатобластома

- **Самое частое ЗНО печени в детском возрасте**
- Врожденная патология
- 95% в возрасте до 4 л., 99% - до 5 л.
- 4-10 л. – «переходные опухоли» схожие с ГЦР
- В 15% случаев – в составе наследственных синдромов



Tomlinson et al., Ped.bl.cancer, 2012

Гепатобластома в составе семейного аденоматозного полипоза

- Риск <1%
- ***mtAPC***
- Чаще nonsense, frame-shift мутации, крупные del
- УЗИ печени и АФП в течении первых 5 лет жизни

Гепатобластома в составе синдрома Беквита-Видеманна

- Риск повышен в 3 р.
- Импринтинг 11p15.5
- УЗИ печени и АФП

Гепатобластома в составе синдрома Ли-Фраумени

- Повышенный риск эмбриональных опухолей
- ***mtTP53***
- УЗИ печени и АФП

Unravelling the genetics of hepatoblastoma: Few mutations, what else?

Journal of Hepatology **2014** vol. 61 | 1202–1204

Marie Annick Buendia*

Hepatoblastoma	Transitional liver tumors	Hepatocellular carcinoma (adults)
2.9 mutations/case (range: 1-7)	27 mutations/case (range: 12-48)	35-75 mutations/case
Wnt pathway *CTNNB1*: 67.5% mostly in-frame deletions *APC*: 1/43 case *AXIN1** 5%	**Wnt pathway** *CTNNB1*: 3/3 in-frame deletions	**Wnt pathway** *CTNNB1*: 10-33% mostly point mutations *APC*: <2% *AXIN1*: 15%
Other genes and pathways • Antioxydant response: *NFE2L2*: 11.7% • Transcription regulators • Chromatin modifiers • Ubiquitin pathway	**Other genes and pathways** • Genetic instability: *RAD17, MSH6, TP53* • Transcription regulators • Chromatin modifiers • Ubiquitin pathway • ***TERT* promoter: 2/3**	**Other genes and pathways** • Antioxydant response: *NFE2L2* + *KEAP1*: 14% • *TP53*: 18-35% • Epigenetic modifiers: (*ARID*, *MLL* families): 32-54% • AKT/mTOR, JAK/STAT, RAS • ***TERT* promoter: 59%**
Genomic alterations <10 CNVs per sample Gains: chr 2, 1q, 8, 6, 12, 17, 20 Losses: chr 4q, 11p	**Genomic alterations** 12-48 CNVs per sample Gains: chr 2, 1q, 6p, 8q, 10, 12, 17, 20 Losses: chr 1p, 4q, 5, 11p, 13	**Genomic alterations** Multiple CNVs per sample More losses than gains Gains: chr 1q, 5, 6p, 7, 8q, 17q, 20 Losses: chr 1p, 4q, 6q, 8p, 13q, 16, 17p, 21

Гепатобластома:

- **Низкий mut уровень**
- ***mtCTNNB1* до 67,5%**
- **Низкий уровень ХН**

Потеря геномной стабильности
mt*TERT* – маркеры агрессивного течения гепатобластомы с клиническими признаками ГЦР


Спасибо за внимание