Molecular perspectives for the treatment of hepatocellular carcinoma

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Abstract

Major advances have been performed in the understanding of genomic dysregulation of hepatocellular carcinoma. A median of 40 to 60 somatic mutations in coding sequence per tumor was identified including 2 to 6 mutations per tumor in genes driving liver carcinogenesis. The main genetic alterations target the key signaling pathways of liver carcinogenesis : telomere maintenance, cell cycle gene, Wnt/beta-catenin pathway, epigenetic modifier gene, oxidative stress pathway, AKT/mTOR and Ras/Raf MAP kinase pathways. A genotype/phenotype classification between these genetic drivers the tumor and patient's features have been also described and was correlated with transcriptomic profiling. These data will be helpful to identify subgroups of HCC that will respond or resist to systemic treatments already used in clinical practice such as tyrosine kinase inhibitors, anti-VEGFR antibody or checkpoint inhibitors and will be useful to identify new therapeutic targets tested in future clinical trials. (Acta gastroenterol. belg., **2020**, 83, **309-312**).

Keywords : molecular classification, hepatocellular carcinoma, targeted therapy, immunotherapy, predictive biomarker, tyrosine kinase inhibitor

Introduction

Hepatocellular carcinoma (HCC) is the first primary liver cancer (85-90%) and the fourth cause of death by cancer worldwide. Most of patients are diagnosed at an advanced stage and will receive systemic therapies (1). A majority of them will experience a primary resistance or develop a secondary resistance to systemic treatments and therefore disease progression. A better understanding of the mechanism of liver carcinogenesis will help to identify new drugs for advanced stages and to understand the determinants of primary and secondary resistance to the approved systemic treatments. This review aims to summarize the recent findings on genomic defects of HCC and how this knowledge could be translated in clinical practice.

1) Molecular classification of hepatocellular carcinoma

Somatic genetic alterations

Next generation sequencing allowed the description of the molecular landscape of HCC with an average of 40-60 somatic alterations detected in protein-coding regions of the genome (2,3,4). Among those alterations, only 2 to 6 occur in "driver" genes that promote liver carcinogenesis. These genes can be classified in 6 main biological pathways, recurrently altered in HCC (Table 1) :

– *Telomere maintenance* with *TERT* promoter mutation in 60% of HCC, *TERT* amplifications in 6% of HCC and 3% of translocations involving *TERT*,

– *Cycle cell regulation* with inactivating mutations of the tumor suppressor gene *TP53* (48%), but also retinoblastoma pathway inactivation by *RB1* mutations (8%), *CDKN2A* homozygous deletions, or *CDKN2A* promoter methylation,

– *Wnt/Beta catenin activation* either by *CTNNB1* activating mutation (35%, encoding ß-catenin), or by inactivating mutations of *AXIN1* (10-15%) or *APC* (2%),

– *Epigenetic dysregulation* with mutations in chromatin remodeling genes such as *ARID1A* (17%) and *ARID2* (18%) or histone methyltransferase genes of *KMT2* family (3%),

– *Constitutive activation of the oxidative stress pathway* by activating mutations of *NFE2L2* (6%) or inactivating mutations of *KEAP1* (4%),

– *Activation of Ras/Raf MAP kinase and PI3K/AKT-mTOR pathways* due to *FGF19* amplification (6%), *TSC1* and *TSC2* inactivation (8%), *VEGFA* amplification (4%), and *RPS6KA3* mutations (9%).

In HCC, 3 main clusters of positive epistatic interactions (associations) have been described, the first between *CTNNB1*, *TERT* promoter, *NFE2L2* and *ARID2* mutations, the second between *AXIN1, ARID1A* and *RPS6KA3* mutations, and finally between *TP53* and *KEAP1* mutations. By contrast, *TP53, CTNNB1* and *AXIN1* tend to be exclusive (2,5).

Several clinical features are also associated with specific genomic alterations. For example, HBV infection is often associated with chromosomal instability and *TP53* mutations, likewise alcohol consumption is

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Table 1. — **Main somatic genomic alterations in hepatocellular carcinoma**

Signaling pathways	Genes	Description of the genomic alteration	%	Clinical/pathological correlation
Telomere maintenance	*TERT*	Promoter mutation	50-60%	Alcohol
		Amplification	5-6%	
		Translocation	2-3%	
Cell cycle	*TP53*	Inactivating mutation /HD	15-48%	HBV/ aflatoxin B1, advanced HCC
	CDKN2A	Inactivating mutation /HD	2-9%	Alcohol, poor prognosis
	RB1	Inactivating mutation /HD	3-8%	Advanced HCC
	ATM	Inactivating mutation	2-6%	
	MYC	Amplification	4-12%	
	CCND1	Amplification	5%	
Wnt/β-catenin	*CTNNB1*	Activating mutation	11-35%	Alcohol
	AXIN1	Inactivating mutation/HD	5-15%	
	APC	Inactivating mutation	1-2%	
Chromatin modifiers	*ARID1A*	Inactivating mutation	5-17%	Alcohol
	ARID2	Inactivating mutation	3-18%	
	BAP1	Inactivating mutation/HD	2-5%	Fibrolamelar "like" features
	KMT2	Inactivating mutation	3%	
Oxydative stress	*NFE2L2*	Activating mutation	3-6%	
	KEAP1	Inactivating mutation	2-4%	
PI3K/AKT-mTOR and map kinase	*FGF19*	Amplification	5-6%	Poor prognosis
	VEGFA	Amplification	4%	
	RPS6KA3	Inactivating mutation	2-9%	
	MET	Amplification	1-2%	
	PTEN	Inactivating mutation/HD	2-3%	
	TSC1 and TSC2	Inactivating mutation	3-8%	
Hepatocytes differentiation	*ALB*	Inactivating mutation	13%	
	APOB	Inactivating mutation	9%	
JAK/STAT	*IL6ST*	Activating mutation	2%	
	JAK1	Activating mutation	1%	
TGFβ	*ACVR2A*	Inactivating mutation	5%	

HD = Homozygous Deletion

associated with *CTNNB1* mutations. Another example is the pathognomonic association between the R249S *TP53* mutation and the exposure to aflatoxin B1, a mycotoxin that contaminates culture in Africa and is responsible of DNA damages that promotes liver carcinogenesis in conjunction with chronic HBV infection (6).

Molecular classification of hepatocellular carcinoma

A classification of HCC in 6 sub-classes (G1 to G6) is currently based on the analysis of genomic, transcriptomic and epigenetic data and is strongly associated with clinical features and risk factors. HCCs are classified into 2 major classes : G1 to G3 subclasses also called the "proliferative" class associated with a poor prognosis and G4 to G6 HCCs classified as "non-proliferative" class (Figure 1) (7). Within the proliferative class (G1 to G3) exists a sub-group of "progenitors" HCCs (G1 subgroup) defined by the overexpression of hepatic progenitor markers (*EPCAM*, *AFP*, *IGF2*) and inactivating mutations of *RPS6KA3* (7,8). Some of G1 HCCs harbored also *BAP1* inactivating mutations together with an activation of protein kinase A and are characterized by a pathological phenotype similar to fibrolamellar hepatocellular carcinoma (9). HCCs from the G3 transcriptomic sub-group are characterized by a histological phenotype called «macrotrabecular massive» associated with a poor prognosis, *TSC1* and *TSC2* mutations, an enrichment in *FGF19/CCND1* amplification and an overexpression of genes related to the cell cycle and nucleus pore (10,11). The "non proliverative class" (G4 to G6) form an heterogenous subclass which includes HCCs with chromosomal stability (Figure 1). The first transcriptomic subgroup (G4 HCC) includes HCCs with a transcriptomic program close to mature hepatocytes program. The second group (G5-G6) is defined by canonical activation of Wnt signalling due to *CTNNB1* mutation with β-catenin accumulation in the nucleus and overexpression of glutamine-synthase, a target gene of this pathway (10).

2) Personalized therapy for HCC

Prediction of response to tyrosine kinase inhibitors and anti VEGFR antibody

Five systemic treatments including tyrosine kinase inhibitors and anti VEGFR2 antibody increased survival compared to placebo in first line (sorafenib, lenvatinib) and second line (regorafenib, cabozantinib and ramucirumab)

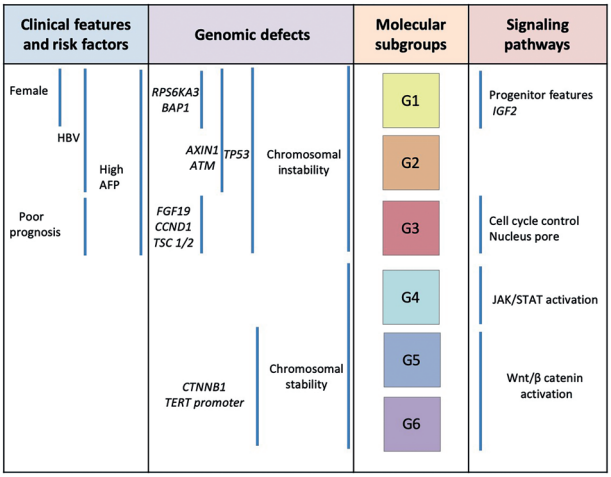

Figure 1. — Molecular classification of hepatocellular carcinoma. We figured the main transcriptomic subclasses of HCC (G1 to G6) and their association with genomic alterations, chromosomal gains and losses, signaling pathways, risk factors and clinical features. Modified from Dhanasekaran R et al (33).

(12,13,14,15). Only one biomarker predictive of treatment response has been validated in a phase 3 randomized controlled trial : serum AFP level (with a cut off > 400 ng/ml) in patients treated by ramucirumab (16). However, the biological meaning of this association is not clear even if molecular analysis showed a higher VEGF level in HCC secreting AFP (17). Biomarkers of response to sorafenib such as *VEGFA* amplification or *FGFR3/FGFR4* amplification or biomarkers of resistance such as activation of Mapk14 (p38α) have been described in retrospective studies (18,19). Circulating proteins (serum LAP TGF-b1, MIP-1a, LOX-1, cystatin B and ANG-1) and circulating microRNA (microRNA 30A, 122,125B, 200A, 374B, 15B, 107, 320B and 645) have been correlated with response to regorafenib (20). However, no robust biomarkers have been validated to predict response or resistance to sorafenib, regorafenib, lenvatinib or cabozantinib in clinical practice.

Prediction of response to immunotherapy

Checkpoint inhibitors alone (antiPD1/PDL1 inhibitor such as nivolumab and pembrolizumab) have failed to overall survival compared to sorafenib in first line and placebo in second line (21). Only the combination of anti-PDL1 antibody (atezolizumab) and anti VEGF antibody (bevacizumab) increased overall survival compared to sorafenib in a recent randomized phase 3 controlled trial (22). In these trials, the retrospective analysis of PDL1 immunostaining was disappointing and failed to predict response to treatment (23). In other cancer types, mutational burden, microsatellite instability or tumor neoantigen were potential predictors of response to checkpoint inhibitors but in HCC no data are currently available about these biomarkers and mutations in mismatch repair genes are relatively uncommon (5). Moreover, transcriptomic immune classifications have been also described in HCC but their role in clinical practice is still unknown (24). Recently, a retrospective clinical study and data from mouse models have suggested that mutation of *CTNNB1* was associated with resistance to immunotherapy (25,26). However, these preliminary data need to be validated in prospective cohort of patients treated by checkpoint inhibitors.

Identification of new therapeutic targets based on genomic analysis

Some biomarkers based clinical trials have been performed in patients with advanced HCC. A phase 2 trial selected patients with HCC with a *KRAS* mutation detectable in the plasma. Among 1318 patients screened, 50 (4.4%) harbored a *KRAS* mutation in circulating tumor DNA and were treated with a MEK inhibitor (refaminitib) +/- sorafenib with a weak tumor response of 6.3% in this highly selected population (27). These disappointing results could be explained by the fact that selection of patients based on plasma sequencing is not specific enough for the detection of HCC harboring *KRAS* mutation (4.4% in the plasma versus 1% in the tumor). Moreover, the drug is probably not effective enough to shut down the pathway activated by *KRAS* mutation.

The first biomarker-based phase 3 randomized controlled trial in advanced HCC has tested a MET inhibitor, tivantinib, compared to placebo in patients with HCC overexpressing MET in immunohistochemistry (28). The trial was negative without any difference between the two arms in term of overall survival. This disappointing result could be explained by the following points : 1) MET overexpression at immunohistochemistry is probably not a biomarker of oncogene addiction to the HFG/MET pathway and 2) tivantinib is finally not a MET inhibitor but acts as a cytotoxic drug (29).

The major obstacle is that the most frequent mutated genes in HCC (*TERT*, *TP53*, *ARID1A* and *CTNNB1*) are currently not druggable. Taking into account the less frequent somatic genetic alterations in HCC (< 10% of the cases), a total of 20 to 25% of advanced HCC harbor a targetable genetic alteration based on drugs available in phase 2 and 3 clinical trials. For example, presence of MET amplification (1% of HCC) was associated with durable tumor response to tepotinib, a specific MET inhibitor (30). Moreover, FGFR4 inhibitor (fisogatinib) have been tested in a phase 1 in 81 patients with advanced HCC stratified on the expression of *FGF19* (31). 17% of tumor response observed in HCC with overexpression of *FGF19* versus 0% in HCC without expression of *FGF19*. Interestingly, secondary mutations were detected in the kinase domain of *FGF19* in patients treated by fisogatinib explaining the occurrence of secondary resistance to the targeted therapy (32).

3) Conclusion

Compared to other solid tumors, we are only at the beginning of personalized treatment of HCC based

on genomic analysis. Implementation of personalized medicine in clinical practice will require well designed translational protocols with tumor and non-tumor biopsies performed both in clinical trials but also in routine. We also need to provide a comprehensive analysis of genomic data together with risk factors, clinical, pathological and imaging features in order to better characterize this disease. This condition, the era of personalized medicine in HCC will begin.

Conflict of interests

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