REVIEW

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Hepatic Oncology

Current issues on genomic heterogeneity in hepatocellular carcinoma and its implication in clinical practice

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Practice points

- Hepatocellular carcinoma (HCC) is a highly heterogeneous disease at the clinical level that can be linked to histological phenotypic and molecular diversity.
- Immunohistochemical and molecular markers, related to prognosis and different pathways of carcinogenesis, have not found their way into treatment management.
- Tumor initiation and progression result from accumulated alterations (copy-number alterations and mutations) in oncogenic drivers and pathways of carcinogenesis.
- Ten mutational signatures (signatures 1A, 1B, 4, 5, 6, 12, 16, 17, 23, 24) are described in HCC.
- Transcriptomic groups are associated with clinical and epi/genetic/genomic features.
- Several molecular profiles have been introduced to potentially predict outcome and improve clinical trial design for targeted treatment.
- The knowledge of molecular intratumor heterogeneity is limited and additional deep sequencing and transcriptome analyses of large HCC series are needed.
- A biopsy-based integrative diagnostic approach including morphology, immunohistochemistry, transcriptomic data, mutational profiles, CNA and methylome analysis could be suitable in the future.
- Multiple-site biopsies, considering intratumor heterogeneity, could refine this process and need to be discussed.

Hepatocellular carcinoma (HCC) is a highly heterogeneous disease leading to a major diversity. Since staging systems are used in patient care, molecular and histopathological features remain to be incorporated in management algorithms. HCC, as other malignant solid tumors, exhibit a complex genetic diversity and genomic instability, driving tumorigenesis. The recent development of deep sequencing techniques has revealed different subgroups of tumors defined by specific patterns of genomic alterations that are related to clinical and histopathological diversity in HCC. Additionally, several genomic defects identified in HCC will be used in the future to develop clinical trial design for tumorized treatment.

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- transcriptomic groups

Hepatocellular carcinoma (HCC) represents the sixth most common cancer (fifth in men and ninth in women) with 782,000 new cases worldwide in 2012. It is the second leading cause of cancer-related mortality, and increases steadily in age-standardized death rates since 1990 [1,2]. HCC is a highly heterogeneous disease at the clinical level and this heterogeneity can be related to the histological phenotypic and molecular diversity (Figure 1). However, HCC is one of the only malignant tumors, for which in the vast majority of cases, no histological proof is mandatory for diagnosis and initiation of cancer-specific treatment, following the clinical practice guidelines of the EASL, AASLD and APASL to diagnose lesions in the background of cirrhosis or chronic hepatitis B virus (HBV) infection [3–6]. In spite of several histopathological, specific immunohistochemical and molecular marker, which have been identified as closely related to prognosis and different pathways of carcinogenesis, incorporation of these markers into treatment management has not taken place.

In times when new personalized treatment options hold the capacity to elevate the level of patient care, new techniques, such as next-generation sequencing (NGS) and large genomic databases (ICGC, TCGA), promise to link tumor genomic profiling with a more individualized management of patients [7–22]. In HCC, several clinical trials for novel molecular treatments have failed in recent years and this may be related to the vast molecular diversity of HCC and the lack of tumor sampling, leading to unselective patient enrollment. In contrast, clinical trial design integrating clinical, histopathological up to specific molecular information of HCC samples could pave the way to personalized treatment and tumor biopsies could lead to specific tumorized treatment.

Clinical-pathological heterogeneity

• Clinical heterogeneity & patient management

HCC is the most frequent primary cancer of the liver (85%) and predominantly affects men at a ratio of 2–4:1 (without cirrhosis approximately 1:1) [23]. At diagnosis, clinical characteristics, such as risk factors, age, local and regional tumor burden, liver function and clinical performance status, strongly vary in accordance to the geographical origin and to the socioeconomic status of patients (Figure 1). Age at diagnosis is indicated to peak at the age of 70 years and to increase

up to 70–79 years in Japan, whereas in central Africa and China patients are younger [3,24–25]. The predominant risk factor in high-incidence regions such as China, Southeastern Asia and Sub-Saharan Africa is chronic HBV infection with or without exposure to AFB1 [23,26]. Chronic hepatitis C virus (HCV) infection is the major risk factor in Japan, where it is diagnosed in 80–90% of the cases [27]. In Europe and North America chronic HCV infection is accompanied by significant alcohol abuse as the most prevalent risk factors [28]. Of note, non-alcoholic steatohepatitis (NASH)-associated HCC is rapidly increasing in numbers in North America [29]. In general, liver cancer is mainly found in less developed regions where over 80% of cases occur [2]. Underlying chronic liver disease occurs in up to 90% and HCC is the most frequent cause of death in patients with cirrhosis [30–32]. Nevertheless, 10% of patients with HCC present without cirrhosis and/or unknown risk factors. To that regard, since cirrhosis is a common co-morbidity, the disease course and overall survival are co-determined by the level of liver impairment.

To summarize, HCC patients present a highly heterogenic clinical background at diagnosis and applying different staging systems, such as Barcelona Clinic Liver Cancer (BCLC) and the Hong Kong Liver Cancer (HKLC), lead to inconsistent prediction of treatment response, disease course and overall prognosis [33]. Furthermore, the implementation of staging systems in clinical practice and a different patient spectrum in Europe/North America (BCLC) and Asia (HKLC) will result in a heterogenic stratification of patients around the world. Profiling of tumor genomic heterogeneity, leading to histopathological heterogeneity and eventually determine the phenotypical heterogeneity, could therefore refine clinical management.

• Histopathological heterogeneity

Since the diagnosis of HCC is achieved by non-invasive criteria in the majority of cases, regular tumor biopsy is not recommended by international guidelines [3–6]. Therefore, 60–70% of nonresectable tumor samples are treated without HCC tissue confirmation leading to a general lack of tissue for precise characterization [34]. However, when imaging is atypical and aiming to distinguish HCC from benign tumors, three immunohistochemical markers, GPC3, HSP70 and GS are recommended by the international

consensus group of hepatocellular neoplasia for tumor-confirmation [35–37]. Whereas some entities simply demonstrate differences in proliferation and differentiation, other HCC present distinct morphological types. For instance, fibrolamellar HCC (FLC) is a very specific morphological subtype of HCC [38–40]. Additional accepted histological patterns are scirrhous, sarcomatoid, inflammatory, or lympho-epithelial-like HCC. Furthermore, up to 5% of HCC samples contain distinct portions of intrahepatic cholangiocarcinoma (ICC) and are referred to as HCC/ICC [41].

Recently, several studies have investigated the clinical impact of immunohistochemical markers within multiple subtypes. Yamashita *et al.* have demonstrated that an EpCAM⁺/AFP⁺ subtype of HCC, representing a high proportion of liver tumor-initiating cells (TIC), exhibits increased tumorigenicity and higher invasiveness [42–44]. In the context of liver TIC, various associated immunohistochemical markers have been introduced such as CK19, CD13, CD24, CD44, CD90 and CD133 [45–53]. Histopathological features of microscopic vascular invasion are independent prognostic factors for progression-free and overall survival in patients with HCC [54,55]. Additionally, it is indicated that the tumor microenvironment could influence tumor characteristics and that the composition of tumor-infiltrating leukocytes, hepatic stellate cells, endothelial cells, hepatoma cells and cancer-associated fibroblasts (CAF) are linked to the histopathological heterogeneity of HCC [56,57].

Recently, further attention was attracted to identify intratumor histopathological heterogeneity [58]. Initially, intratumor heterogeneity in HCC was reported by Kenmochi *et al.* and An *et al.*, who have demonstrated intratumor variations in differentiation and proliferation in two thirds and in up to half of HCC tissue samples, respectively [59,60]. Taking into account morphological and immunohistochemical features Friemel *et al.* demonstrated intratumor heterogeneity in at least one immunohistochemical characteristic in 87% of cases [58]. In addition, 50% of their HCC samples presented different expression patterns of CK7 and GS within one tumor [58].

Alongside the role of the tumor microenvironment, inter- and intratumor histopathological variability are linked to driver mutations in regards to clonal evolution and to the concept

of stem cell-like TIC [56,61–63]. With recent advances in NGS light has been shed on the issue of cancer driver genes and associated oncogenic pathways.

Molecular heterogeneity

• Intertumor heterogeneity

Tumor initiation and progression result from accumulated alterations in oncogenic drivers and pathways finally leading through clonal evolution to intertumor molecular heterogeneity (Table 1) [7–13,15–17,22,64]. Several differences in alteration rates and activation of oncogenic pathways are assumably dependent on heterogeneous clinical characteristics (risk factors, underlying liver disease, or disease stage). Therefore, results of original papers depend very much on the tissue they have included in their studies.

Overall, activating *TERT* alterations (promoter mutation, focal amplification, or HBV insertion) are the most frequent alterations occurring in HCC [11,18–20,22]. Moreover, *TERT* promoter mutations are the earliest genetic alterations occurring during malignant transformation of cirrhotic nodules in HCC or during hepatic adenoma-carcinoma transition in association with *CTNNB1* mutations [11,18–20]. Recurrent activating mutations of *CTNNB1* (11–37%) are the second most common alterations observed in HCC (Table 1); more frequent in HBV- and alcohol-related tumors [8,11,15,22,64]. Additionally, recurrent inactivating mutations of β -catenin inhibitors, *AXIN1*, *APC* or *ZNRF3*, lead to an activating Wnt signaling in up to 66% of all liver cancers (Table 1) [11,22]. Inactivating mutations in *TP53* are also highly frequent (20–52%), more predominantly in HBV-related HCC and associated with poor prognosis [8–9,11,15,22,64]. Beyond that, characteristic R249S somatic mutations in *TP53* are linked to AFB1 exposure and are clustered in HBV infected patients in high endemic regions such as Sub-Saharan Africa [22]. Notably, alterations in *CTNNB1* and *TP53* are significantly exclusive from each other; they define two different clusters of HCC [22]. Several genes encoding chromatin remodeling factors are also frequently mutated in HCC. Among them, *ARID1A* and *ARID2* are the genes most recurrently inactivated in HCC cohorts around the world (8–17%) [7–9,11,22]. Significant association to alcohol [22] or to non-HBV/non-HCV cases in Japan [11] is highlighted, implying an important tumor suppressor role of the SWI/SNF chromatin remodeling

Figure 1. Inter- and intra-tumor heterogeneity. Hepatocellular carcinoma is a highly heterogeneous disease dependent on geographical origin, risk factors, underlying liver disease, and the disease stage.

Figure 1. Inter- and intra-tumor heterogeneity (cont.). This clinical heterogeneity is associated to molecular diversity (CNA) leading in part to histopathological diversity. Additionally, intratumor heterogeneity is present and a biopsy-based integrative diagnostic approach including histopathology, transcriptomic classification, mutational profiling, CNA, epigenetic profiling and calculation of prognostic gene scores could improve patient management in terms of tumorized treatment.

CNA: Copy number alteration.

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complex in nonviral HCC. Mutations in additional cancer drivers have been identified: *ATM* (up to 19%), *ALB* (up to 13%), *RPS6KA3* (up to 10%), *CDKN2A* (up to 9%), *IGSF3/10* (up to 9%), *JAK1* (up to 9%), *RBI* (up to 8%), *KEAP1* (up to 8%), *TSC2* (up to 5%), *FGF19* (up to 5%) and *ARID2* (up to 4%) (Table 1). From the clinical point of view, alcohol as a risk factor is further associated with alterations in *SMARCA2*, *RBI* and homozygous deletions in the *CDKN2A* locus [22]. Alterations in *IL6ST* and less alterations in *TERT* are significantly found in cases without known etiologies [22].

Recurrent somatic copy number alterations (CNA), leading to chromosomal losses and gains, were identified at 1p, 4p-q, 6q, 8p, 13p-q, 16p-q, 17p, 21p-q, 22q and at 1q, 5p, 6p, 8q, 17q, 20q, Xq, respectively [8,11,15,22,64]. Moreover, frequent homozygous deletions inactivating *AXIN1*, *CDKN2A/B*, *CFH*, *IRF2*, *MAP2K3*, *PTEN*, *PTPN3*, *RBI*, *RPS6KA3* were found, whereas recurrent focal amplifications were described associated with an overexpression of *CCNE1*, *FGF3/4/19/CCND1*, *JAK3*, *MET*, *MYC*, *TERT* and *VEGFA* [8,11,15,22,64]. Integrating CNA and frequent mutations in 161 putative cancer driver genes, pointed out frequent alterations of 11 major oncogenic pathways, including from the most to the least frequent, alterations in telomere maintenance, Wnt signaling, PI3K/mTOR pathway, p53 pathway, MAP kinase pathway, hepatic differentiation, epigenetic regulation, chromatin remodeling, oxidative stress, JAK/STAT pathway and TGF- β signaling (Table 1) [22]. Nevertheless, noticeable differences are observed among HCC cohorts, for example, alterations in the p53 pathway ranging from 33 to 72% and in PI3K/mTOR signaling ranging from 12 to 51%, reflecting heterogeneity related to geographical and risk factor distributions [11,22]. Along malignant transformation from dysplastic macronodules to poor prognosis HCC, we have found an increasing number of gene alterations and CNA per stage [22]. *TERT* promoter mutations were early events in hepatocarcinogenesis

whereas focal amplifications of the *FGF3/4/19/CCND1* locus were significantly associated with poor prognosis HCC [22]. Common alterations found in hepatocellular adenoma (*HNF1a*, *IL6ST*) have not been discovered in HCC in nonfibrotic liver tissue, implying that malignant transformation of hepatic adenomas is not frequent [22]. Nevertheless, the presence or absence of underlying liver disease may affect growth patterns.

Molecular diversity in HCC is also observed at the transcriptomic level, showing several subgroups of tumors characterized by a specific profile of gene expression [14,65–67]. Significant associations linked these transcriptomic subgroups with clinical and epi-/genetic/genomic features. The noteworthy integrative approach, including gene expression, genetic alterations and clinical features, remains to be performed in HCC related to risk-factor profiles. Moreover, thanks to multinational approaches HCC-subtypes have been molecularly classified to date, for example, three groups in a cohort of 78 FLC samples are reported as proliferative, inflammatory and unannotated subclass of FLC, taking into account multiple molecular technics [68].

Novel exciting genomic classifications are proposed by mutational signatures, linking exposure to risk factors to specific patterns of nucleotide mutations. As described by Alexandrov *et al.*, a computational framework allows deciphering distinct patterns of somatic mutations in a set of cancer samples as mutational signatures based on 96 possible nucleotide triplets including the 3' and 5' nucleotides of substitution sites [14,69–71]. In a pancancer study, eight mutational signatures (signatures 1A, 1B, 4, 5, 6, 12, 16, 17) were described in HCC [70]. Associations for signatures 1A, 1B (age), 4 (tobacco) and 6 (defects in mismatch repair) are assumed, whereas for signatures 5, 12, 16 and 17 genotoxic causes are unknown. A recent study also found mutational signatures associated with ancestry groups (European, Japanese, and US Asian) without relation to specific risk factors (HBV, HCV,

Table 1. Alterations in oncogenic drivers and major oncogenic pathways.

Study (year)	Sample size	Sequencing technique	Most frequently altered cancer driver	Most frequently altered oncogenic pathway	Major risk factors	Ref.
Fujimoto <i>et al.</i> (2012)	27 + 120	Whole genome + validation set	<i>TP53</i> (52%) <i>ATM</i> (19%) <i>IGSF10</i> (15%) <i>CTNNB1</i> (11%) <i>ARID1A</i> (11%)		HCV (52%) HBV (41%)	[9]
Guichard <i>et al.</i> (2012)	24 + 125	Whole exome + validation set	<i>CTNNB1</i> (33%) <i>TP53</i> (21%) <i>ARID1A</i> (17%) <i>AXIN1</i> (15%) <i>RPS6KA3</i> (10%) <i>CDKN2A</i> (7%)	Wnt pathway (49%) p53 pathway (33%) Chromatin remodeling (23%) PI3K/Ras pathway (13%) Oxidative stress (6%)	Alcohol (37%) HBV (24%) HCV (19%) NASH (5%)	[8]
Huang <i>et al.</i> (2012)	10 + 100	Whole exome + validation set	<i>TP53</i> (27%) <i>ARID1A</i> (13%) <i>SAMD9L</i> (6%) <i>ARID2</i> (4%)		HBV (100%)	[7]
Cleary <i>et al.</i> (2013)	87	Whole exome	<i>CTNNB1</i> (23%) <i>TP53</i> (20%) <i>CPA2</i> (9%) <i>IGSF3</i> (9%) <i>KEAP1</i> (8%)		HBV (43%) HCV (21%) Alcohol (11%)	[16]
Kan <i>et al.</i> (2013)	88	Whole genome	<i>TP53</i> (35%) <i>CTNNB1</i> (16%) <i>LRP1B</i> (11%) <i>JAK1</i> (9%) <i>AXIN1</i> (5%)	Wnt pathway (63%) JAK/STAT pathway (46%) Apoptosis pathway (46%) p53 pathway (43%)	HBV (92%)	[15]
Ahn <i>et al.</i> (2014)	231	Whole exome	<i>TP53</i> (32%) <i>CTNNB1</i> (23%) <i>RB1</i> (8%) <i>AXIN1</i> (7%) <i>SELPLG</i> (5%) <i>FGF19</i> (5%)	p53 pathway (37%) Wnt pathway (37%) Chromatin remodeling (34%) Cell cycle (22%) PI3K/Ras pathway (12%)	HBV (72%) HCV (10%)	[64]
Totoki <i>et al.</i> (2014)	608	Whole genome + whole exome	<i>TERT</i> (54%) <i>CTNNB1</i> (31%) <i>TP53</i> (31%) <i>ARID1A</i> (8%) <i>AXIN1</i> (6%) <i>TSC2</i> (5%)	p53 pathway (72%) Telomere maintenance (68%) Chromatin remodeling (67%) Wnt pathway (66%) PI3K/mTOR pathway (45%) Oxidative stress (19%)	HCV (42%) HBV (23%)	[11]
Schulze <i>et al.</i> (2015)	235	Whole exome	<i>TERT</i> (60%) <i>CTNNB1</i> (37%) <i>TP53</i> (24%) <i>ARID1A</i> (13%) <i>ALB</i> (13%) <i>AXIN1</i> (11%) <i>CDKN2A</i> (9%)	Telomere maintenance (60%) Wnt pathway (54%) PI3K/mTOR pathway (51%) p53 pathway (49%) MAP kinase pathway (43%) Hepatic differentiation (34%) Epigenetic regulation (32%) Chromatin remodeling (28%)	Alcohol (41%) HCV (26%) NASH (18%) HBV (14%)	[22]

HBV: Hepatitis B virus; HCV: Hepatitis C virus; NASH: Nonalcoholic steatohepatitis.

nonviral) [11]. Additional exome data demonstrated two novel mutational signatures (signatures 23 and 24) [22]. Signature 23 was identified in a woman presenting black deposition of mineral silica in the nontumor liver tissues

suggesting exposition to an unidentified risk factor. Signature 24 was identified in patients from Africa, showing typical R249S mutation in *TP53*. Thus, this signature is characteristic of the AFB1 exposure [22].

• Intratumor heterogeneity

To understand the biological basis of intratumor heterogeneity, the evolutionary history of tumors has to be taken into account. Mutational signatures reflect the diversity of exogenous and endogenous impacts on genome stability occurring during cancer evolution, leading finally to the outgrowth of major clones and/or several subclones [70]. Moreover, intratumor heterogeneity can result of different modes of branched tumor evolution: during linear evolution mutagenic processes accumulate sequentially over time. In case of two genetically and geographically distinct minor clones within one tumor, branched cancer evolution can progress independently, synergistic, or antagonistic between subclones within one tumor [72].

Regarding HCC, our knowledge of molecular intratumor heterogeneity is limited [58,73] and we need additional data in large series of tumors analyzed using deep sequencing and transcriptome experiments. The knowledge of intratumor heterogeneity will be particularly important to interpret the results of resistance to treatment and identifying subclones selected by drugs.

Consequences for clinical practice

The fast growing knowledge about tumor genomic heterogeneity in HCC will lead to an individual molecular profiling. Therefore, like in other types of tumors, it is believed that precise molecular profiling will impact on clinical treatment decision for HCC patients.

• Molecular diversity according to prognosis

Several molecular signatures have been introduced to potentially predict outcome in patients with HCC (Table 2).

Yamashita *et al.* demonstrated that gene expression profiles of stem cell-like TIC marker (EpCAM, CD90 and CD133) may determine the clinical outcome in patients with HCC [80]. Nault *et al.* introduced a five-gene mRNA (*HNI*, *RAN*, *RAMP3*, *KRT19* and *TAF9*) score predicting disease-specific survival, independent of clinical or histopathological features, in 748 patients worldwide [21]. Macro- and micro-scopic vascular invasion are significant predictors for HCC recurrence after resection and it has been associated with a 35-gene-expression signature in 214 patients with HCC [81]. Ye *et al.* identified a molecular signature associated to intrahepatic metastases and poor outcome [88]. Another study investigated the role of gene signatures

in development of metastases and identified a 161-gene signature that was validated in 386 HCC patients [78]. Finally, Okamoto *et al.* introduced a 36-gene-expression profile predicting multicentric HCC in nontumor liver tissue with single or multiple tumor nodules [90].

Several groups analyzed miRNA patterns and their association to prognosis. Applying a 67-miRNA signature Barry *et al.* showed a significant association with HCC recurrence after liver transplantation in 64 patients [77]. Budhu *et al.* found a 20-miRNA tumor signature identifying patients who are at higher risk of developing metastases and predicting overall survival [93].

Recently, Villanueva *et al.* validated a 36-DNA-methylation signature and demonstrated accurate poor survival prediction in 304 patients with HCC [74]. Moreover, the 36-DNA-methylation signature is associated to an mRNA-based signature significantly indicating hepatic progenitor cells [74].

In conclusion, several promising signature models have been introduced and prospective studies have to validate the reproducibility in treatment stratification, for example, curative versus palliative approach.

• Targeted therapies adapted to the molecular diversity

Treatment options are limited in patients with HCC, particularly for patients in BCLC stage C, because until today sorafenib is the only approved systemic agent. In spite of a broad arsenal of small molecular inhibitors and monoclonal antibodies, multiple efforts of implementing new targeted treatments into clinical practice failed in recent years [94–101]. In part, inadequate patient selection and a ‘diluted treatment effect’ after unselective patient enrollment are thought to be the reasons. Hence, it is believed that future clinical trial designs need to implement the individual molecular profile to allocate patients to an adequate targeted treatment.

Recently, two large-scale NGS studies have schematized a broad panoramic view of putative cancer drivers and major pathways involved in hepatocarcinogenesis and pointed out potential therapeutic targets in accumulated 853 patients with HCC [11,22]. Molecular profiles (CNA and mutations) revealed 11 altered major pathways: telomere maintenance, WNT/ β -catenin, PI3K/AKT/mTOR, TP53/cell cycle, MAP kinase, hepatic differentiation, epigenetic

Table 2. Molecular signatures potentially predicting outcome in patients with hepatocellular carcinoma.

Study (year)	Signature	Molecular feature	Sample size	Major risk factors	Ref.
Villanueva <i>et al.</i> (2015)	36-DNA-methylation marker score	Methylome	221 + 83 (validation)	HBV, HCV, alcohol	[74]
Wei <i>et al.</i> (2013)	30-miRNA signature	miRNA	60 + 50 (validation)	HBV	[75]
Kim <i>et al.</i> (2012)	65-gene-based risk score	mRNA	139 + 292 (validation)	HBV	[76]
Barry <i>et al.</i> (2012)	67-miRNA HCC recurrence signature	miRNA	69	HCV, HBV, NASH	[77]
Roessler <i>et al.</i> (2010)	Metastasis gene signature	mRNA	247 +139 (validation)	HBV	[78]
Lee <i>et al.</i> (2004)	Proliferation cluster of genes	mRNA	91	HBV, HCV, alcohol, hemochromatosis, Wilson disease	[67]
Lee <i>et al.</i> (2006)	Hepatic progenitor cell signature	mRNA	139	HBV, HCV, alcohol	[79]
Boyault <i>et al.</i> (2007)	G1-6 transcriptome classification	mRNA	120	HBV, HCV, alcohol	[14]
Nault <i>et al.</i> (2013)	Five-gene score	mRNA	314 + 434 (validation)	HBV, HCV, alcohol, hemochromatosis	[21]
Yamashita <i>et al.</i> (2013)	EpCAM ⁺ /CD90 ⁺ gene expression score	mRNA	102	HBV, HCV, alcohol	[80]
Yamashita <i>et al.</i> (2009)	EpCAM ⁺ gene expression score	mRNA	156	HBV	[42]
Hoshida <i>et al.</i> (2009)	S1-3 expression-based subclasses	mRNA	603	HBV, HCV	[66]
Minguez <i>et al.</i> (2011)	35-gene signature of vascular invasion	mRNA	79 + 135 (validation)	HCV	[81]
Toffanin <i>et al.</i> (2011)	miRNA-based classification of HCC	miRNA	89	HCV	[82]
Kurokawa <i>et al.</i> (2004)	20-gene-based prediction score	mRNA	100	HBV, HCV	[83]
Iizuka <i>et al.</i> (2003)	Statistical Pattern Recognition method	mRNA	33 + 27 (validation)	HCV, HBV	[84]
Wang <i>et al.</i> (2007)	57-member molecular gene signature	mRNA	25	HBV, HCV	[85]
Woo <i>et al.</i> (2008)	HCC recurrence signature	mRNA	65	HBV	[86]
van Malenstein <i>et al.</i> (2010)	Seven-gene signature associated with hypoxia	mRNA	272 + 91 (validation)	HCV, HBV, alcohol, NASH, AIH, PBC	[87]
Ye <i>et al.</i> (2003)	Metastatic HCC gene signature	mRNA	67	HBV	[88]
Yoshioka <i>et al.</i> (2009)	Prediction score of early recurrence	mRNA	139	HCV, HBV	[89]
Okamoto <i>et al.</i> (2006)	Gene signature for multicentric HCC	mRNA	40	HCV	[90]
Chiang <i>et al.</i> (2008)	Molecular classification of HCV-associated HCC	mRNA	109	HCV	[91]
Jiang <i>et al.</i> (2008)	miRNA prognostic signature	miRNA	54	HCV, alcohol, HBV, hemochromatosis	[92]
Budhu <i>et al.</i> (2008)	20-miRNA tumor signature	miRNA	131 + 110 (validation)	HBV	[93]

regulation, chromatin remodeling, oxidative stress, IL-6/JAK/STAT and TGF- β . Within these pathways multiple oncogenes or tumor suppressor genes are potentially prone to targeted treatment. The French study reported that 28% of patients harbored at least one damaging alteration, potentially targetable by an FDA approved [22]. Moreover, 86% of the

patients are potentially treatable by a drug, screened in clinical trials Phase I–III [22]. The most attractive target genes are involved in the PI3K/AKT/mTOR and MAPK pathways, since growth factor receptors and their ligands are well-suitable for tyrosine kinase inhibitors and antibodies. Only a small proportion of patients harbored alterations for a unique

potential gene: *FLT3* (6%), *FGF3/4/19* (4%), *PDGFRs* (3%), *EPHA4* (3%), *VEGFA* (1%), *HGF* (3%), *EGFR* (1%), *FGFRs* (1%), *KIT* (1%), *MET* (1%), *TEK* (1%), *ERBB2* (<1%), *KDR* (<1%) [22]. Hence, these findings underline the necessity of precise patient stratification for adequate clinical trial design. Despite frequent activating alterations of the PI3K/AKT/mTOR pathway (51%) and mutations of *MTOR* (2%), the mTOR inhibitor everolimus also failed in a Phase III trials as second line treatment [22,97]. Described by Sawey *et al.*, focal amplification of the *FGF19/3/4/CCND1* locus is an effective predictor for anti-FGF19 treatment in HCC [102]. Subsequent high-throughput sequencing studies found amplifications of *FGF19* in up to 6% in human HCC samples promising targeted treatment in a subgroup of patients with HCC [11,22,64]. Of note, one study reported *FGF19* amplifications are associated to poor prognosis patients, implying poor prognosis patients also for the control arm in clinical trial design [22]. At the end of the day, direct linkage of tumor genomic profiling with individualized patient management is far more complex. Extensive biological and pharmacological studies will be needed to understand how driver genes can be efficiently targeted and how to overcome resistance. Clinical trial design needs to integrate clinical, histopathological and specific molecular information of HCC samples to pave the way to personalized

treatment. Moreover, taken into account intra-tumor heterogeneity, additional multiple-site biopsies could lead to specific tumorized treatment. Nevertheless, current data are from resection specimens. Therefore, further prospective studies, including tissue samples of biopsies, will answer these important questions in the future.

Conclusion & future perspective

Up until today, stratified molecular information has not been incorporated into staging systems utilized in HCC. A biopsy-based integrative diagnostic approach, including morphology, immunohistochemistry/-fluorescence, transcriptomic expression data, mutational profiles, CNA and methylome analyses, could be suitable in the future (Figure 1). Moreover, multiple-site biopsies, considering intratumor heterogeneity, could refine this process and need to be debated (Figure 1). Prospectively, this valuable knowledge could improve patient stratification for adequate enrollment in clinical trials.

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