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Linking Molecular Classification of Hepatocellular Carcinoma and Personalized Medicine: Preliminary Steps

Augusto Villanueva, M.D.¹, Sara Toffanin, Ph.D.^{2,3}, and Josep M. Llovet, M.D.^{1,2}

¹BCLC Group. Liver Unit. CIBERehd. IDIBAPS. Hospital Clinic, Barcelona, Spain

²Mount Sinai Liver Cancer Program. Division of Liver Diseases, Mount Sinai School of Medicine, New York, USA

³Department of Experimental Oncology, Fondazione IRCSS, Istituto Nazionale Tumori, Milan, Italy

Abstract

Recent advances have improved our understanding of the molecular pathogenesis of hepatocellular carcinoma (HCC). The development of high-throughput technologies able to simultaneously investigate thousands of genes (SNP-array, gene expression microarray, etc.) has opened a new era in translational research. However, obtaining a molecular classification of HCC remains a striking challenge. Different investigators attempted to classify patients according to their liver cancer molecular background, a feature that will path the way for trial enrichment and personalized medicine. Currently, HCC can be classified in molecular classes according to Wnt- β catenin pathway activation, proliferation signature activation (associated with chromosomal instability), and other subgroups. In parallel, the first-time ever positive results of a phase III trial in advanced HCC with the multikinase inhibitor sorafenib have encouraged this approach. The current review summarizes the molecular classifications of HCC reported, analyzes the status of targeted therapies tested in clinical trials, and evaluates feasibility of personalized medicine approaches in HCC.

Keywords

Liver cancer; genomics; signaling pathway; molecular classification; personalized medicine; molecular targeted therapies; sorafenib

EPIDEMIOLOGY OF HEPATOCELLULAR CARCINOMA

(Supongo que queries hablar de molecular epidemiology of HCC, pero no hay nada)

Hepatocellular carcinoma (HCC) is the leading cause of death among cirrhotic patients, and it has become a global health problem¹. Based on recent estimations, close to 625,000 new cases of HCC will be diagnosed worldwide every year. HCC mortality ranks third among all cancers, only behind lung and colon cancer^{2,3}. Incidence rates of HCC have been closely related to viral hepatitis. Hepatitis B virus (HBV) infection is the main risk factor for HCC in Eastern Asia and Africa^{4,5}. Accordingly, population-based studies of massive hepatitis B vaccination have shown robust decreases in HCC incidence⁶. Several studies have shown that aflatoxin B exposure has a synergistic effect with HBV infection for the development of

HCC. In Western countries and Japan, hepatitis C virus (HCV) infection is still the main risk factor⁷.

Prognosis of HCC is dreadful. Less than 40% of patients are eligible for potential curative treatments, and systemic therapies such as standard chemotherapeutic agents are not efficacious in HCC based on randomized trials^{8–10}. Hence, new therapeutic approaches are urgently needed. Also, with the advent of surveillance programs, the scientific community has experienced a switch in the type of tumors detected, and the medical interventions potentially effective for them. It is hoped that in clarifying the genomics and signaling pathways implicated in hepatocarcinogenesis, new therapeutic targets will be developed.

HCC is a very complex tumor from a molecular perspective¹¹. Unlike most human malignancies, HCC usually develops in the context of inflammation and organ injury. This fact, added to the number of different etiologies responsible for liver damage (e.g. viral hepatitis, alcohol, etc.), warrants high molecular variability and precludes any simplistic approach. For this reason, it is relevant to figure out which are the predominant molecular mechanisms for a given HCC so that its selective and effective blockade will result in the control of the disease (*personalized medicine*)¹². This concept comprises the study of patients as biologic individuals, allowing customization of medical interventions according to their unique genomic background.

The complexity of the human genome is far beyond what was expected after its complete decoding in the early 2000's. New confounding factors such as alternative splicing, mRNA stability, epigenetic gene silencing, etc, have increased the output diversity after DNA transcription. Unexpectedly, since the accomplishment of the Human Genome Project, there have not been major research breakthroughs with substantial clinical implications. Also, less than 10% of published associations between DNA alterations and disease have been validated by independent researchers¹³. Association studies require notable numbers of patients so that small changes in DNA with implications in cancer development can be detected. These drawbacks have diminished the early enthusiastic predictions about the clinical achievements after implementation of personalized medicine. Nevertheless, certain landmark studies leave plenty of room for optimism (i.e. prophylactic surgery in women with BRCA1/2 mutations¹⁴, selective response to tyrosine kinase inhibitors in patients with lung cancer and EGFR amplifications¹⁵, etc.).

Herein, we review the current status and feasibility of personalized approaches for the treatment of HCC. For that purpose, we will first summarize the molecular classifications proposed for HCC; and second, we will evaluate present and future of molecular target therapies for this neoplasm.

(que tiene que ver este titulo con el texto?)

In most human malignancies, clinical classifications are essential for prognosis assessment and treatment planning. Also, they are useful to unify criteria and allow unbiased comparisons between groups of patients within research protocols. In the last decade, and parallel to advances in molecular oncology, genomic parameters have been progressively included in cancer classifications¹⁶. Unlike traditional clinical information (i.e. tumor size, presence of vascular or lymph node invasion, etc.), molecular data accounts for the biological background of the neoplasm. This is two-fold relevant; first, the type and quantity of molecular aberrations in a tumor might be surrogates of clinical aggressiveness. Some examples are summarized in Table 1. Secondly, molecular aberrations may represent new targets for drug development. For example, HER2/Neu amplifications in breast cancer and EGFR overexpression/mutations in non-small cell lung cancer (NSCLC) identify a subgroup of patients with better response to trastuzumab and in some instances erlotinib,

respectively^{17,18}. As a result, tailored therapeutic regimes based on molecular data are progressively being introduced in clinical routine (Table 2).

Golub et al. pioneered molecular cancer classification using gene expression monitoring by DNA microarrays in acute myeloid leukaemia¹⁹. In this milestone study, gene expression data were able to accurately predict cancer classes, independent of previous biological knowledge of the samples. Thereafter, several studies following the same methodology confirmed the prognostic value of gene signatures in different malignancies, including HCC^{20–22}. Supervised and unsupervised learning methods are widely used to create gene signature profiles²³. However, the application of molecular signatures as predictors of survival has been seriously criticized. Michiels²⁴ found a worrisome rate of misclassification in 7 studies analyzed, as well as high instability in the signatures described. Five out of the seven largest published studies at that time addressing cancer prognosis using gene expression microarrays were unable to classify patients better than chance. The reasons reported for such unstable prediction were related to design issues. In a training-validation approach, the training set identifies the molecular signature and the validation set is used to estimate the degree of misclassification. In principle, Michiels recommended large sample sizes for the training sets and the use of validation by repeated random sampling. Clearly, the methodology used for data management in traditional biomedical research cannot be simply translated into this type of studies where thousands of variables are analyzed simultaneously. Thus, initial concerns related to data processing jeopardized the valuable information provided by genome-wide expression studies.

There is no molecular classification of HCC. Several clinical staging systems have been proposed to predict HCC prognosis but only the BCLC staging system has gained wide recognition among scientific societies^{25–27}. Still it does not incorporate biological information of the tumor. The accumulation of genetic aberrations driving cirrhotic liver to cancer appears to be a multi-step process. This transformation includes histopathological lesions that range from liver cirrhosis to dysplastic nodules (low- and high-grade), but the underground molecular mechanisms involved in this process are not fully understood. During the preneoplastic stage, there is an upregulation of mitogenic pathways leading to the selection of certain clones of dysplastic cells. These clones, organized as dysplastic nodules surrounded by fibrotic connective tissue, will acquire a malignant phenotype when exposed to different genomic alterations, including structural aberrations in chromosomes, microsatellite instability, etc^{11,28}. It is thought that this mitogenic phenotype leads to the activation of pathways involved in pro-apoptosis and cell cycle arrest, which provide with survival benefit to dysplastic. The final step in this malignant transformation includes pathways related to angiogenesis and the capacity for migration and invasion²⁹.

MOLECULAR CLASSIFICATION OF HCC

Considerable efforts have been made to obtain a molecular classification of HCC (Table 3). However, the overwhelming genomic complexity of HCC has rendered this goal as a major challenge. Whole-genome based analysis of DNA aberrations^{30,31} or gene expression^{32,33} have been frequently used to define molecular subgroups. These high-throughput technologies have afforded researchers a unique opportunity to integrate large amount of molecular information with clinical parameters. Unfortunately only two studies have comprehensively integrated data from different platforms^{31,34}. These multidimensional approaches complement *integrative functional genomics*, a term that refers to the integrated analysis of gene expression data sets from different species (human HCC, mouse models and HCC cell lines³⁵). Within this general framework, Lee et al. were able to identify a new subtype of HCC patients who shared a gene expression pattern with fetal hepatoblasts^{33,36}.

Preliminary attempts to classify HCC patients according to tumor molecular background were unidimensional, and based on genome-wide gene expression data³⁷. Initial studies compared gene expression between HCC and non-tumoral tissue, and were later improved by gene expression analysis of the whole hepatocarcinogenic process (including cirrhotic tissue and preneoplastic lesions)^{38,39}. Ye et al. studied 67 primary and metastatic HCC by an unsupervised hierarchical clustering algorithm interrogating 9,180 genes⁴⁰. Interestingly, they failed to obtain significant differences in gene expression between primary and metastatic lesions. This feature is seminal to understand and characterize clonality in multinodular HCC, and it has been addressed elsewhere⁴¹. However, Ye et al. found that primary metastasis-free HCC had a gene expression profile markedly different from that of primary HCC with metastatic lesions, also with differences in patient survival. The 153-gene model provided a robust signature that correctly classified 100% of the training samples during cross-validation. All these data implied that genes favoring metastasis progression are initiated in the primary tumors, and authors argued that primary HCC with metastatic potential may be evolutionary distinct from metastasis-free primary HCC. Furthermore, stringent analysis of expression data unleashed osteopontin as a relevant mediator in metastatic HCC.

Similarly, another study using high-density oligonucleotide microarray reported a 12-gene signature able to predict early intrahepatic HCC recurrence with an accuracy of 93%⁴². Subsequently, and using a different technology (adaptor-tagged competitive PCR) in a training-validation approach, Kurokawa et al. studied gene expression in 60 HCC patients⁴³. Authors selected a 20-gene signature out of 92 candidate genes able to predict early recurrence (< 2 years). The recurrence signature ("poor signature") was validated in an independent cohort of 40 patients with a predictive accuracy close to 73%. The "poor signature" was an independent predictor of recurrence (HR 3.82, 95% IC 1.44–10.10, $p=0.007$). As noted, accuracy rates were lower than in previous studies, and unfortunately there were no common genes among these reports. Prognosis modeling using gene expression analysis was conducted in the Laboratory of Experimental Carcinogenesis (NCI, Bethesda, USA). In 2004, Lee et al. analyzed global gene expression patterns in 91 HCC to define the molecular characteristics of HCC, and to test the prognostic value of expression profiles³². Unsupervised hierarchical clustering revealed 2 classes of HCC patients characterized by significant differences in survival (30 vs 84 months in class A and B, respectively). After supervised analysis, authors found 406 genes whose expression was significantly informative of length of survival. Expression of typical cell proliferation markers was greater in class A. Both classes shared significant similarities when compared with non-tumoral tissue. This suggested that tumors in class A accumulated additional oncogenic alterations on top of a common HCC gene expression signature. However, data reported in this study did not exclude the possibility that different mechanisms were primarily responsible of two molecularly different types of tumors, referred as "etiological footprint" by the authors. This hypothesis was further explored in a second study where they tested if global gene expression analysis of human HCC would identify subtypes of HCC derived from different cells (hepatocytes and hepatic progenitor cells³³). For that purpose, Lee et al. integrated data from gene expression of 139 human HCC, 39 rodent HCC (from 5 five different tumor mouse models), and rat fetal hepatoblasts and adult hepatocytes. They uncovered a new subclass of HCC ($n=22$ patients, "hepatoblast signature, HB") that co-clustered with rat fetal hepatoblasts (Table 3). This signature was independently associated with both recurrence and worse survival, and it was enriched of JUN and FOS activity. The HB signature also displayed markers of hepatic progenitor cells (KRT7, KRT19 and VIM). Whether this signature is consequence of malignant transformation of mature hepatocytes with dedifferentiation remains unclear.

Chromosomal alterations in HCC have been surveyed by comparative genomic hybridization (CGH)⁴⁴, array-based CGH and high-density single nucleotide polymorphism (SNP) arrays⁴⁵. Despite several technical improvements, recent array-based studies predominantly reported single copy gains and losses of entire chromosome arms, rather than recurrent high-level amplifications or homozygous deletions smaller than 5 Mb. This result suggests that multiple genes residing on the same chromosome arm may be under simultaneous selection, due to the synergistic proliferative advantages conferred by copy number alterations to these genes¹¹. Recently, Katoh et al. proposed a molecular classification of HCC based on chromosomal alteration profiles detected with array-based CGH³⁰. They identify 2 distinct patterns (clusters A and B) after analyzing 87 HCCs. Chromosomal alterations were observed more frequently in cluster A (1q, 6p, 8q gains, and 8p losses were almost exclusive to this cluster), and patients in this cluster had worse survival. Both clusters were further subdivided in 3 subclasses each. Subclusters B2 and A3 harbored amplification in 17q11.2–25.3, locus of the *RPS6KB1* gene, a downstream molecule of the mTOR signaling pathway. Based on this finding, authors hypothesize that patients within these subclusters could be ideal candidates for mTOR inhibitors. Likewise, patients in subcluster A1 were distinctively characterized by amplifications in 1q and/or 6p. Among several potential oncogenes in these regions, VEGF seems to be an ideal candidate for selective target since several studies support the role of VEGF in HCC^{46–49}. Also, positive results of a phase III clinical trial with a tyrosine kinase inhibitor against VEGFR – sorafenib-in advanced HCC reinforce the potential role of anti-VEGFR therapy in HCC⁵⁰.

The study of HCC with a systems biology approach assumes that cancer deregulates a complex network of gene and proteins. Therefore, multi-parametric measurements assessing changes in this equilibrium will facilitate accurate prognostic and therapeutic modeling in a personalize medicine scenario. A pioneer study following a multidimensional approach analyzed 137 HCC patients that were characterized for mutations in p53, Axin1 and β -catenin in conjunction with evaluation of allelic loss using 335 microsatellite markers³¹. Based on these data, authors were able to classify HCC patients in two groups: First, a chromosomal instability class, defined by high FAL index (Fraction of evaluable autosomal arms demonstrating allelic deletion), and frequent mutations in p53 and Axin1. This group was significantly enriched with HBV infection, poor differentiated tumors, and patients had worse prognosis, similarly to cluster A in Katoh's classification³⁰. The second group, chromosomal stability, had low FAL index and mutations in β -catenin. Patients in this class tended to have larger tumors and to be non-HBV infected. The same researchers recently reported an update of this classification expanding the parameters evaluated³⁴. Based on mutations in *PI3KCA*, *EGFR*, *KRAS*, *NRAS* and *HRAS*, promoter methylation, gene expression evaluated with microarray, qRT-PCR and protein analysis using western blot and immunohistochemistry (IHC), they identify 6 groups (G1-G6). G1-G3, included previously in the chromosomal instability group, were characterized by frequent allelic loss, mutations in Axin1 and p53, activation of Akt signaling and overexpression of IGF2. Young females with HBV infection and high serum AFP were the most relevant clinical features of G1-G2. Group 3 was enriched in genes associated with cell cycle. On the other hand, groups G5-G6 had lower rates of allelic loss (chromosomal stable), frequent β -catenin mutations and nuclear localization of β -catenin, what suggested activation of Wnt signaling. A gene signature formed by 16 genes was able to accurately allocate patients in each class. However, unlike their previous report, authors were unable to detect differences in survival within these six groups.

Recently, the Mount Sinai Liver Cancer Program performed an integrative genomic analysis on 103 HCV-related HCC using annotated data from SNP-array, gene expression profiling, mutation analysis and IHC (unpublished data)⁵¹. Five molecular subclasses were identified after unsupervised analysis of HCC transcriptome. Three of them further refine classes

previously reported (activation of β -catenin³⁴ and proliferation³⁶ classes, interferon-related class). Also, this study detected a new class characterized by gains in chromosome 7.

Cellular ability to metastasize is a hallmark of cancer⁵². As mentioned before, gene expression studies have tried to classify HCC patients according to their predisposition to present intrahepatic metastasis^{40,42}. Few investigations addressed the contribution of host genetic background (i.e. microenvironment) in this process. Budhu et al. compared the gene expression profiles of 115 non-tumoral adjacent liver tissues from HCC patients with and without venous or distant metastasis. Strikingly, a signature of 454 genes generated from adjacent non-tumoral tissue significantly distinguished patients with and without metastasis. Authors narrowed down to 17 the number of genes required to accurately classify patients according to metastasis potential, and they named it “refined liver microenvironment venous metastasis signature”. Genes in this signature were mostly related to the immune/inflammatory response. In addition, this signature showed that the predicted metastatic group had a significant shorter survival and time to recurrence.

Remarkably, there is certain degree of class aggregation across the different molecular classifications of HCC proposed so far. As an example, in three independent studies there is high concordance in a subclass of HCC patients characterized by dysregulation of genes related to cell proliferation (G1-G3 classes in Boyault’s³⁴ study, Proliferation class in Chiang’s study⁵¹ and HA class in Lee’s study³³). Also, it is consistent throughout different reports the existence of a group of patients with recurrent chromosomal aberrations, suggesting chromosomal instability. However, there is still contradictory data among studies, and a significant group of patients remain repeatedly unclassified.

TARGETED THERAPIES IN HCC: INSIGHTS FROM A PERSONALIZED MEDICINE APPROACH

Assuming a personalized medicine perspective, when clinically applicable tools are available to accurately classify HCC patients according to their tumor molecular background, it would be feasible to customize therapies. Unfortunately, in some studies the use of molecular therapies has by-passed this mandatory understanding of the molecular portrait of cancer. The first evidence of molecular prediction of response was reported with the fusion kinase bcr-abl and response to imatinib in chronic myeloid leukemia. Following this landmark discovery, the response to trastuzumab in patients with breast cancer harboring amplifications of HER2/nu represent the most characteristic example of molecular prediction of response to targeted therapies in solid tumors. Other less compelling examples are the prediction of response to erlotinib in patients with NSCLC with EGFR amplifications/mutations. HCC is a particular case in oncology. Unlike most of the solid tumors, deaths in HCC can be related to the tumor or to liver dysfunction. Hence, it is essential to correctly design clinical trials in HCC. In order to capture antineoplastic effect in an investigational drug, HCC, and not liver dysfunction, has to dictate response to treatment. Recently, a panel of experts has published some guidelines for the design of clinical trials in HCC⁵³. The introduction of biological agents as therapeutic options in oncology has changed the traditional paradigm to measure the antitumoral effect of new agents in the clinical trial scenario. The most important issues are regarding which should be the primary and secondary endpoints in controlled clinical trials. Obviously, objective response is a weak surrogate of activity in phase II trials, since in most cases, the predominant effect of these compounds is basically cytostatic. In fact, bevacizumab has shown a survival benefit in patients with liver metastases, as has erlotinib in advanced lung cancer and sorafenib in renal cancer, even when objective responses were less than 10%. Therefore, it is mandatory to select primary endpoints able to capture the effects associated with stabilization of the disease for clinically relevant periods. These endpoints need to have a time-to-event format.

Progression-free survival is the best endpoint in most human malignancies, but it is not appropriate in HCC since it combines two types of events: progression and death due to any etiology. Therefore, it is recommended the inclusion of time-to-progression (period between the inclusion in the study and the radiological progression of the disease) as the primary endpoint in phase II trials, and survival as the endpoint in phase III trials⁵³.

Several studies have tried to establish the activation status of different signaling pathways in HCC. Data suggest activation of numerous oncogenic signaling routes in HCC (i.e. EGF, IGF and HGF signaling, RAS/MAPK, Akt/mTOR pathway, NF-Kappa β , TGF- β signalin, Wnt- β Catenin, etc^{11,54}). Consequently, several drugs have been developed to block these pathways at different levels (Fig 1). However, since pathway activation is not uniform in all HCC patients, it would be desirable to know *a priori* which pathway is activated in every patient. This concept is tightly linked to the molecular classification of HCC since some molecular classes are led by the activation of certain pathways (e.g. β -catenin in class G5-6 of Boyault³⁴).

Different phase II and III trials have evaluated molecular therapies in HCC^{50,55-61}. Most of them used drugs that block EGF signaling (erlotinib) and angiogenesis (bevacizumab), and there is only one study that analyzed association regimes with traditional chemotherapy⁶⁰. Eckel et al. reported a phase II trial testing imatinib in 17 HCC patients⁵⁶. Authors reported a median survival of 12 weeks and 33% of patients with stable disease. Interestingly, only one of the patients in this trial was positive for PDGFR and none was positive for c-Kit. It is difficult to know if imatinib could play a role in HCC if almost none of the patients in this trial had activation of two of the most relevant targets of this drug. Two phase II trials have been conducted with erlotinib in HCC^{58,59}. The fact that the rate of stable disease in both studies is quite different suggests that the population selected for both trials was not uniform. Both studies analyzed EGFR receptor status with IHC, and both agreed in the absence of predictive power of this marker for response to erlotinib. However, significant differences in time to progression in patients treated to sorafenib according to the phosphorylation status of ERK, a known downstream molecule of B-RAF were reported⁵⁵. Again, it is unclear if the selection of IHC for total EGFR as biomarker of response to erlotinib is accurate enough in HCC. In other malignancies, the presence of amplifications or phosphorylation of EGFR (hallmark of activation of the receptor) and other downstream molecules predicted response to tyrosine kinase inhibitors⁶².

A major breakthrough occurred in 2007 in the targeted therapy field in HCC. An oral multikinase inhibitor (sorafenib) with activity against several tyrosine kinase (VEGFR2, PDGFR, c-Kit receptors), and serine/threonine kinases (B-RAF, p38) was able to significantly increase survival in patients with advanced HCC^{50,63}. This was the first phase III trial to show positive results with a target therapy in HCC, and it was also the first systemic therapy to increase survival in advanced HCC. Previously, a phase II clinical trial involving 137 patients with advanced HCC exposed that sorafenib induced stable disease for 4 months in 35% of the patients with an overall median survival of 9.7 months⁵⁵. Partial response rate was less than 10%.

The randomized phase III double-blind placebo-controlled clinical trial conducted in patients with advanced HCC treated with sorafenib has shown improvement in survival of 3 months in patients with advanced HCC, which was not only statistically significant, but also clinically meaningful⁵⁰. The study was stopped at the second planned interim analysis because of survival advantages favoring the treatment arm. The median overall survival was 10.7 months with sorafenib and 7.9 months with placebo (hazard ratio for death, 0.69; 95% confidence interval, 0.55 to 0.87; $P < 0.001$). The magnitude of the difference-reflected by the HR of 0.69-compares well with the differences obtained with other targeted therapies in

colorectal cancer (cetuximab, bevacizumab), renal cancer (temsirolimus), breast cancer (trastuzumab) and lung cancer (erlotinib). Median time to progression was 5.5 months with sorafenib *versus* 2.8 months with placebo (hazard ratio 0.58; 95% confidence interval 0.45 to 0.74; $P < 0.001$). Seven patients (2.3%) in the sorafenib group and two patients (0.7%) in the placebo group achieved a partial response. Recently, the drug has been approved both by FDA and EMEA for the treatment of HCC.

Sorafenib targets two of the main pathways involved in hepatocarcinogenesis by blocking angiogenesis (VEGFR2 and PDGFR) and cell proliferation through Ras/MAPK signaling (b-Raf). An exhaustive analysis of the trial data suggests that the majority of patients receiving sorafenib achieve some clinical benefit. It is biologically plausible that a drug able to block different molecules will benefit a wide range of patients since many signaling pathways will be target simultaneously. Thus, sorafenib is *de facto* acting as a multitarget therapy in HCC. Additional challenges will be to combine this drug with other targeted therapies blocking signaling networks untouched by sorafenib, particularly in those patients with chromosomal instability that could have several signaling pathways aberrantly activated. Potential combination therapies are summarized in Figure 1.

CONCLUSIONS AND FUTURE PERSPECTIVES

The development of a molecular classification of HCC is crucial. Until the molecular pathogenesis of HCC is completely understood, it would be complicated to achieve high rates of curation with targeted therapies. Many progresses have been made in this regard, and preliminary reports using genome-wide approaches confirm the high genetic heterogeneity of HCC. It seems that a subgroup of patients have activation of pathways related to cell proliferation (IGF signaling, ras/MAPK and mTOR signaling) and differentiation (Wnt- β -catenin). Etiology of the underlying liver disease also seems to confer specific chromosomal aberrations. It is also clear that tumor microenvironment plays a major role in HCC intrahepatic metastasis. Probably, gene expression studies will soon allow predicting risk for HCC development based on the gene signature in cirrhotic tissue. Fortunately, sorafenib has opened the trail for target therapies in HCC. Certainly, the conjunction of precise knowledge of the molecular classes of HCC, well-designed clinical trials, and effective blockade of specific pathways with new therapies will definitely implement personalized medicine for the clinical management of HCC.

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Abbreviations

CGH	Comparative genomic hybridization
FISH	Fluorescence in situ hybridization
HCC	hepatocellular carcinoma
HBV	Hepatitis B virus
HCV	Hepatitis C virus

IHC	immunohistochemistry
LOH	Loss of heterozygosity
NSCLC	non-small cell lung cancer
RT-PCR	real-time polymerase chain reaction
SNP	Single nucleotide polymorphism
TKR	Tyrosine Kinase Receptor

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Figure 1. Signaling pathways and targeted therapies in HCC. This figure summarizes some of the most relevant signaling pathways analyzed in human HCC (RAS/MAPK, Akt/mTOR, Wnt-βcatenin and Hedgehog). Also, it shows different drugs in clinical and preclinical development for the specific blockade of these pathways.

Table 1

Diagnostic and prognostic biomarkers in human malignancies.

Author	Molecular Alteration	Method	Type of Marker	Clinical Significance
Ithier G ⁶⁴	BRCA1 mutation	DNA sequencing	Diagnostic	High risk for development breast cancer
Powell SM ^{65,66}	APC mutation	DNA sequencing	Diagnostic	High risk for development of colon cancer
Lips CJ ⁶⁷	RET mutation	DNA sequencing	Diagnostic	High risk for development of medullary thyroid cancer
Berwick M ⁶⁸	CDKN2A mutation	DNA sequencing	Diagnostic	High risk for development of melanoma
Llovet JM ⁶⁹	3-gene signature (Glypican-3, LYVE1, Survivin)	Real-Time PCR (RT-PCR)	Diagnostic	Molecular diagnosis of early HCC (accuracy rates: 85–95%)
Robinson HM ⁷⁰ , Radich J ⁷¹	Translocation t(9;22)	Fluorescence In Situ Hybridisation (FISH). RT-PCR for Bcr-Abl fusion transcript. Sequencing	Diagnostic and Prognostic	Marker of chronic myeloid leukemia (CML) and prognostic of high risk for relapse after bone marrow transplantation
Hurd DD ⁷²	Translocation t(15;17)	FISH. RT-PCR for PML-RARA fusion transcript. Sequencing	Prognostic	Favorable outcome of patients affected of promyelocytic leukemia
Kenny FS ⁷³	Cyclin D1 overexpression	Northern Blotting. IHC	Prognostic	Earlier relapse and shorter overall survival of ER+ breast cancer patients
Huncharek M ⁷⁴ , Slebos RJ ⁷⁵	K-Ras mutation	Sequencing	Prognostic	Shortened survival in NSCLC and in lung adenocarcinoma
Jen J ⁷⁶	Loss of chromosome 18q	Microsatellite markers	Prognostic	Poor outcome in patients with stage II colorectal cancer
Offit K ⁷⁷	Rearrangement of the bcl-6 gene	Southern Blot hybridization	Prognostic	Favorable clinical outcome in diffuse large-cell lymphoma
Van de Vijver MJ ⁷⁸	70-gene signature	Microarray analysis	Prognostic	Outcome predictor in young patients with breast cancer
Paik S ⁷⁹	21-gene signature (recurrence score)	Reverse-transcriptase-polymerase-chain-reaction	Prognostic	Distant recurrence in tamoxifen-treated patients with node-negative, estrogen-receptor-positive breast cancer

Table 2

Potential biomarkers of response to molecular therapies in human cancer.

Author	n	Tumor type	Molecular predictor of response	Drug	Response to treatment	Status
Early Breast Cancer Trialists Collaborative Group ⁸⁰	37000 (metaanalysis of 55 clinical trials)	Breast	Estrogen receptor expression	Tamoxifen	Improvements in 10-year survival after 5-years treatment with Tamoxifen	First line of therapy for the treatment of ER+ breast cancer
Piccant-Gebhart MJ ⁸¹	1694 (Phase III)	Breast	Amplification of HER2/Neu	Transtuzumab	Improvement of disease-free survival	Approved for treatment of metastatic HER2+ breast cancer
Hirsch FR ^{82, 83}	1692 (Phase III)	NSCLC	FISH for EGFR (high copy number level)	Gefitinib	Objective response rate and TTF (time to treatment failure)	Approved for the treatment of advanced lung cancer as third-line therapy for non-small-cell lung cancer
Tsao M ¹⁵	427 (Phase III)	NSCLC	IHC for EGFR	Erlotinib	Improved responsiveness assessed according to the Response Criteria In Solid Tumors (RECIST)	Approved for the treatment of advanced NSCLC
Sawyers CL ⁸⁴	260 (Phase II)	CML	FISH for chromosomal translocation t(9;22)	Imatinib mesylate	Hematologic responses in 52% of patients and cytogenetic responses in 16% of patients	Approved by FDA for the first-line treatment of chronic myeloid leukemia
Van Oosterom AT ⁸⁵	40 (Phase I)	GIST	IHC for c-Kit	Imatinib mesylate	Inhibition of tumor growth in 35 patients, 19 confirmed partial responses	Systemic therapy with Gleevec is currently approved for the treatment of c-kit-positive unresectable and metastatic GIST
Gordon MS ⁸⁶	123 (Phase II)	Ovarian	Enzyme-linked immunosorbent assay (ELISA) for p-HER2	Pertuzumab	Better response rate in patients positive for p-HER2	Phase II and III clinical trials in breast and ovarian cancers ongoing
Abou-Alfa GK ⁵⁵	137 (Phase II)	HCC	IHC for p-ERK	Sorafenib	Longer time to progression in p-ERK positive patients	Approved for patients with hepatocellular carcinoma after phase III trial (SHARP). Molecular markers studies under development

Table 3

Relevant proposals of molecular classifications of HCC

Author	Technology applied	n	Molecular classes	Genomic alteration	Clinical correlation
Laurent-Puig, 2001 ³¹	Mutation analysis and LOH	137	Chromosome instability	High FAL [*] . Mutations in Axin1 and p53. LOH in 1p, 4q, 16p, 16q, 13q, 9p, 6q	HBV infection, poor differentiation and prognosis
Boyault, 2007 ³⁴	Mutation analysis, LOH, promoter methylation, gene expression microarray, RT-PCR, western blot and IHC	120	Chromosome stability	High FAL. Mutations in β -catenin. LOH in 8p	Non-HBV infection. Larger tumors.
			G1	Mutations in Axin1. LOH in 16q and 16p Mutations in PI3KCA and p53. Akt activation Mutations in p53. LOH 5q, 21q and 22q Non-specific alterations Mutations in β -catenin, hypermethylation of E-cadherin	HBV infection, young women and high AFP HBV infection. Hemochromatosis Satellites
			G2		
			G3		
			G4		
			G5	Chromosome stability	
			G6		
Chiang, 2007 (unpublished data) ⁵¹	SNP-array, gene expression microarray, mutation analysis and IHC	103	β -catenin	Mutations and nuclear translocation of β -catenin Activation of TKR-dependent pathways (IGF and mTOR). Chromosomal instability Overexpression of genes induced by IFN (STAT, IFI6, etc) Gains in chromosome 7. Overexpression of c-Met, COBL, SMO, etc. Non-specific alterations	Vascular invasion and high levels of AFP Lower recurrence rate Higher rates of early recurrence (<2 years)
			Proliferation		
			IFN-response		
			Poly 7		
			Unannotated		
Ye, 2003 ⁴⁰	Gene expression microarray	67	Non-metastatic	Osteopontin overexpression	Worse survival
			Metastatic		
Kurokawa, 2004 ⁴³	Adaptor-tagged competitive PCR	100	Good signature	Known genes included in the signature: CDH1, IGF2R, RB1, NRG2	Significant worse survival and higher rates of early recurrence
			Poor signature		
Lee, 2004 ³²	Gene expression microarray	91	Cluster A	Overexpression of cell proliferation markers and genes related to ubiquitination and sumoylation	Worse survival
			Cluster B		
			Cluster B		
Lee, 2006 ³³	Gene expression microarray	139 ^{\$}	Cluster A	Overexpression of AP-1 transcription factors (FOS, JUN) and markers of hepatic oval cells (KRT7, KRT19 and VIM)	Worse survival Worst survival
			HC subtype		
			HB subtype		
Budhu, 2006 ⁸⁷	Gene expression microarray	200 ^{&}	Inflammation/immune response cluster A	A 17 gene signature discriminates samples with or without metastasis. Genes included in the signature were mostly related to the immune/inflammatory response	Signature that predicted metastatic group had a significant shorter survival and time to recurrence
			Inflammation/immune response cluster B		

Author	Technology applied	n	Molecular classes		Genomic alteration	Clinical correlation
Katoh, 2007 ³⁰	Array-based CGH	87	Cluster A	Subcluster A1	Amplifications in 1q, 6p and/or 8q, gains 7 p/q and losses on 8p and 13q. Amplifications in 8q, losses on 8p and 13q, gains on 1q. Candidate oncogene: c-myc Gains on 6p, 7 p/q, 8q, 17q, and losses on 8p and 13q	Worse survival
				Subcluster A2		
				Subcluster A3		
			Cluster B	Subcluster B1	Non-specific alterations Amplifications in 17q and losses on 10q Gains in 9 p/q	Suggested activation of mTOR pathway.
				Subcluster B2		
				Subcluster B3		

* Fraction of evaluable autosomal arms demonstrating allelic deletion

[§] Besides these 139 human HCC samples, 39 mouse HCC, rat fetal hepatoblasts and adult hepatocytes were also profiled

^ℳ Gene expression studies were performed on adjacent non-tumoral tissue. Signatures were generated from tumoral microenvironment

Integrar algun mensaje de las Tablas 1 y 2 en el texto.