Integrative Genomic Analysis Identifies the Core Transcriptional Hallmarks of Human Hepatocellular Carcinoma

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Abstract

Integrative genomics helped characterize molecular heterogeneity in hepatocellular carcinoma (HCC), leading to targeted drug candidates for specific HCC subtypes. However, no consensus was achieved for genes and pathways commonly altered in HCC. Here, we performed a meta-analysis of 15 independent datasets (n = 784 human HCC) and identified a comprehensive signature consisting of 935 genes commonly deregulated in HCC as compared with the surrounding non-tumor tissue. In the HCC signature, upregulated genes were linked to early genomic alterations in hepatocarcinogenesis, particularly gains of 1q and 8q. The HCC signature covered well-established cancer hallmarks, such as proliferation, metabolic reprogramming, and microenvironment remodeling, together with specific hallmarks associated with protein turnover and epigenetics. Subsequently, the HCC signature enabled us to assess the efficacy of signature-relevant drug candidates, including histone deacetylase inhibitors that specifically reduced the viability of six human HCC cell lines. Overall, this integrative genomics approach identified cancer hallmarks recurrently altered in human HCC that may be targeted by specific drugs. Combined therapies targeting common and subtype-specific cancer networks may represent a relevant therapeutic strategy in liver cancer. Cancer Res; 76(21); 6374–81. ©2016 AACR.

Introduction

Liver carcinogenesis is a long process associated with multiple risk factors that contribute to hepatocellular carcinoma (HCC) heterogeneity, for example, viral hepatitis, alcohol abuse, metabolic disorders, and obesity (1). Most HCCs develop in the setting of chronic liver disease associated with cycles of tissue destruction and regeneration that result in the activation of numerous signaling pathways and the accumulation of genomic alterations. Lately, next-generation sequencing approaches highlighted the signaling pathways and the accumulation of genomic alterations.

Assessment of tumors and 3 main HCC subtypes were identified (6). However, transcriptional profiling of recurrent transcriptional alterations in HCC has been largely neglected, so far. Failure to define a robust common transcriptional fingerprint in HCC may have resulted from technical variabilities and/or from the inherent HCC molecular heterogeneity. The latter even raises the question about the existence of a substantial core expression signature in HCC (7). However, genomic characterization of HCC mouse models demonstrated that various oncogenic pathways could generate similar expression profiles at the tumor stage, suggesting that a common HCC signature probably exists in humans (8). This observation prompted us to perform a meta-analysis of publicly available human HCC datasets that were generated over a period of more than 10 years. Starting from raw microarray data and by using the same analysis algorithms to circumvent technical variabilities, our aim was to define a universal and comprehensive transcriptional signature in HCC and to determine whether this signature could be useful to identify clinically relevant drug candidates.

Materials and Methods

Analysis of microarray datasets

The meta-analysis was performed using gene expression datasets available from open databases, namely Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo/) and ArrayExpress (www.ebi.ac.uk/express/). Twenty-eight liver-oriented datasets were retrieved through a systematic review of the literature on PubMed and queries of the microarray databases using PubMed identifiers or keywords associated with human liver carcinogenesis and large-scale gene expression profiling, for example, HCC, microarray, and gene profiling (Supplementary Table S1). Before performing the analysis, all microarray platforms (n = 19) were re-annotated using the Database for Annotation, Visualization and Integrated Discovery (DAVID; https://david.ncifcrf.gov/; ref. 9). In total, 24,085 nonredundant annotated genes were present at least in 1 of 28 retrieved microarray datasets. Statistical analysis of microarray data was performed using R-based BRB-ArrayTools as

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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applied and differentially expressed genes between the tumor experiments were performed (50 μmol/L) after 48 and 72 hours of treatment with DMSO, trichostatin A was evaluated using a PrestoBlue cell viability reagent (Invitrotable provided by cMap and a review of the literature. Cell viability treat the cells was determined on the basis of the detailed results purchased from Sigma-Aldrich. All molecules were solubilized in LY294002, rapamycin, resveratrol, sorafenib, and suberoylanilide

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Data mining of the core HCC gene signature

Several tools dedicated to the discovery of specific enrichments for biologic functions or canonical pathways were used, including Enrichr algorithm (http://amp.pharm.mssm.edu/Enrichr) and Ingenuity Pathway Analysis (IPA). IPA was also used to examine the functional association between differentially expressed genes and to generate the highest significant gene networks using the IPA scoring system. Gene Set Enrichment Analysis (GSEA) was performed by using the Java-tool developed at the Broad Institute (Cambridge, MA; www.broadinstitute.org/gsea) as previously described (11). Connectivity map (cMap) algorithm was used to link gene expression signatures with putative therapeutic molecules (10, 12). Briefly, from the permuted results cMap table, we focused on negative enrichments to retain only the perturbagens (i.e., molecules) that potentially reverse the expression of genes included in the core HCC signature. Only the perturbagens with a permuted \( P < 0.003 \) and a number of replicate more than 5 were retained.

Cell culture

A panel of 6 liver cancer cell lines was purchased from ATCC (www.lgstandards-acc.org), including SNU-475 (ATCC CRL-2236, grade II–IV/V), SNU-449 (ATCC CRL-2234, grade II–III/IV), SNU-423 (ATCC CRL-2238, grade III/IV), SNU-387 (ATCC CRL-2237, grade IV/V), HepG2/C3A (ATCC CRL-10741), and SK-Hep-1 (ATCC HTB-52). ATCC performed cell lines authentication by STR DNA profiling. The impact of selected molecules was evaluated within 6 months after receipt. Cells were grown in a RPMI-1640 medium supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% FBS. Cultures were performed at 37°C in a 5% CO₂ atmosphere. α-Estradiol, LY294002, rapamycin, resveratrol, sorafenib, and suberylanilide hydroxamic acid (SAHA, also known as vorinostat) were purchased from Santa Cruz Biotechnology. Trichostatin A was purchased from Sigma-Aldrich. All molecules were solubilized in a DMSO solution: the concentration of each molecule used to treat the cells was determined on the basis of the detailed results table provided by cMap and a review of the literature. Cell viability was evaluated using a PrestoBlue cell viability reagent (Invitrogen) after 48 and 72 hours of treatment with DMSO, trichostatin A (1 μmol/L), α-estradiol (1 μmol/L), vorinostat (50 μmol/L), sorafenib (2 μmol/L), rapamycin (2 μmol/L), LY294002 (50 μmol/L), and resveratrol (500 μmol/L). Independent culture experiments were performed (n = 4 independent biologic replicates; n = 4 technical replicates for each biologic replicate). The significance of differences in cell viability between experimental conditions was determined by a 2-tailed nonparametric Mann–Whitney test. For the microarray experiments, the concentrations were optimized to induce 50% cell mortality after a 72-hour drug exposure, to allow the extraction of nucleic acids from the remaining viable cells. Accordingly, cells were treated with trichostatin A (0.6 μmol/L), α-estradiol (1 μmol/L), vorinostat (3.7 μmol/L), sorafenib (0.65 μmol/L), rapamycin (2 μmol/L), LY294002 (45 μmol/L), and resveratrol (166 μmol/L).

Gene expression profiling

Total RNA was purified from SNU-423 cells at 50% confluence with an miRNAeasy kit (Qiagen). Genome-wide expression profiling was performed using the Low-Input QuickAmp Labeling Kit and human SurePrint G3 8 × 60K pangenomic microarrays (Agilent Technologies) as previously described (10). Starting from 150 ng total RNA, amplification yield was 7.0 ± 1.3 μg cRNA and specific activity was 18.2 ± 2.3 pmol Cy3 per μg of cRNA. Gene expression data were processed using Feature Extraction and GeneSpring softwares (Agilent Technologies). Microarray data have been deposited in NCBIs GEO and are accessible through GEO Series accession numbers GSE79246 and GSE85257.

Results

Generation of a compendium of gene expression profiles in human HCC

The initial step of the study was to assemble well-annotated human HCC gene expression profiles. From the main public databases (i.e., GEO and ArrayExpress), 28 liver-oriented datasets were retrieved including 1,657 HCC gene expression profiles from a total of 3,047 (Fig. 1A). A detailed characterization of HCC datasets is provided in the Supplementary Table S1. Of these 28 datasets, 15 (MDS1–MDS15) included both HCC and surrounding nontumor tissues and thus were relevant to derive a core HCC signature defined as a set of genes differentially expressed between HCC and surrounding nontumor tissues. Importantly, the gene expression profiles were generated from various microarray platforms (i.e., from academic or industrial sources, including several updated contents). Consequently, variations in both the number and the nature of interrogated gene features between platforms greatly impede the integrative analysis of the datasets. To overcome this issue, all the platforms (n = 19, Fig. 1A) were reannotated by using the DAVID database (9). In total, 24,085 annotated genes were present at least in one microarray dataset (Fig. 1A).

Identification of a comprehensive 935-gene HCC signature linked to recurrent early genomic alterations in liver carcinogenesis

By using the same normalization, filtration, and statistical algorithms, a set of genes significantly deregulated between HCC and surrounding nontumor tissues was identified [\( P < 0.01; \) false discovery rate (FDR) < 1%; n = 15 datasets; Fig. 1A]. The so-called universal core HCC signature consisted of 935 genes significantly deregulated in more than 50% HCC datasets and included 41% upregulated genes (Fig. 1B; Supplementary Table S2). Clustering analysis based on the expression of these genes in 15 datasets revealed a clear transcriptional homogeneity in the investigated tumors (n = 784 HCC, Fig. 1B). Validating the gene selection, the 935-gene HCC signature included well-known HCC biomarkers (e.g., GPC3, PEG10) or HCC suppressor genes (e.g., DLC1;
Supplementary Table S2). In addition, chromosomal mapping of genes included in the 935-gene HCC signature highlighted a bias in the distribution of up- and downregulated genes at specific locations known to be frequently altered in HCC (Fig. 1C; Supplementary Table S3). Thus, upregulated genes were significantly ($P < 0.001$) enriched in 1q and 8q whose amplifications were previously reported as the earliest events that occur in human hepatocarcinogenesis (13, 14). Similarly, downregulated genes were enriched in loci frequently subjected to early deletions in HCC (e.g., 4q). These specific locations also correlated with the deregulation of HCC-associated genes (e.g., LAMC1 at 1q31, RAD21 at 8q24, or ALB at 4q13). Altogether, these observations demonstrated that transcriptional changes identified in the 935-gene HCC signature significantly correlated with early and recurrent structural genomic alterations linked to stepwise HCC carcinogenesis in human.
The 935-gene HCC signature covers well-established cancer hallmarks

Data mining of the 935-gene HCC signature (Fig. 1D and E; Supplementary Tables S4 and S5) identified gene networks that reflect a definite transcriptional reprogramming associated with previously described hallmarks of cancer cells, as exemplified thereafter (15).

Sustained proliferation and evasion to growth suppressors. Gene networks and Gene Ontology analysis clearly demonstrated that active cell proliferation is the most prominent feature within the 935-gene HCC signature (Fig. 1E). Thus, numerous cell cycling genes were upregulated (Supplementary Table S2), including cyclins (e.g., CCNB1, CCNE2), cyclin-dependent kinases and inhibitors (e.g., CDK1, CDK4, CDKN2A), cell-cycle and cell division checkpoints (e.g., BUB1, MAD2L1, NDGR, RAN), together with well-established proliferation markers (e.g., PCNA, MKI67). Accordingly, numerous genes related to DNA replication were induced (e.g., CDC6, MCM2-4, RFC4). These observations coincided with the induction of proproliferative pathways and growth factors (e.g., EGR3, MAPK6, YWHAH, FIBP) and the repression of negative feedback regulators (e.g., PINK1, DUSP1).

Genome instability, oxidative stress, and apoptosis resistance. A sustained proliferation phenotype generally associates with DNA damages, accumulation of mutations, and genome instability, which ultimately lead to apoptosis (15). Interestingly, the 935-gene HCC signature highlighted gene deregulations that clearly sign mechanisms of DNA damages associated with DNA hyperreplication together with resistance to cell death (Supplementary Table S2). Thus, several genes essential for DNA double-strand break repair (e.g., RAD21, RUVBL2) and nucleotide excision repair (e.g., ERCC3, FEN1, OGG1) were induced. Notably, DNA glycosylase OGG1 is involved in the excision of 8-oxoguanine, a mutagenic base byproduct that occurs as a result of exposure to reactive oxygen species. Further implying oxidative stress in this process, key regulators of redox homeostasis were repressed in the 935-gene HCC signature (e.g., CTH, CBS, NFE2L2, PRDX4). Besides, several inducers of apoptosis were repressed (e.g., CRADD, D APK1), whereas genes encoding proteins that prevent apoptotic cell death were induced (e.g., BIRC5, DAD1).

Metabolic reprogramming. Deregulation of metabolism-associated genes (e.g., lipid, carbohydrate and amino acid metabolisms) was a prominent feature in the 935-gene HCC signature, in agreement with metabolic changes observed in cancer cells during tumor onset and progression (16). This was particularly noticeable for downregulated genes involved in liver-specific metabolisms (Fig. 1D; Supplementary Tables S4 and S5), including those encoding acute-phase plasma proteins (e.g., A2M, ALB, CP), components of complement and coagulation cascades (e.g., C5-9, CFB, F2), or detoxication enzymes (e.g., ADH1A, CYP2E1). While such enrichment may reflect a loss of hepatocyte differentiation accompanying tumor development, other deregulations affecting key enzymes of bioenergetics and biosynthesis may be directly linked to a specific reprogramming of cellular metabolism (Supplementary Table S2). As example, ACLY and CS were recurrently upregulated in HCC. ACLY encodes ATP citrate lyase, the primary enzyme for the synthesis of acetyl-CoA, a major intermediate for biosynthetic pathways including lipogenesis. CS encodes the citrate synthase, a key enzyme in the tricarboxylic acid cycle that contributes to lipogenesis by enhancing the conversion of glucose to lipids. While lipogenesis was enhanced, genes encoding key components of lipid catabolism were repressed, including genes involved in the mitochondrial fatty acid β-oxidation pathway (e.g., ACADM, ACADL, ECHS1). In addition, we observed a shift toward the downregulation of genes involved in gluconeogenesis (e.g., PCK1, FBP1) that may contribute to enhance the rate of glycolysis in HCC cancer cells.

Induction of angiogenesis. Fueling proliferative and metabolically active tumor cells frequently correlates with an enhanced angiogenesis to support nutrient supply (15). Accordingly, the 935-gene HCC signature included several angiogenesis-related genes. As example, PLG encoding plasminogen was strongly repressed in almost all HCC (Supplementary Table S2). Of note, plasminogen is activated by proteolysis and converted to plasmin, an activator of matrix metalloproteases, and angiostatin, a potent inhibitor of angiogenesis. Similarly, AMOTL2 encoding an angiostatin like 2 protein was repressed. Angiomotin is known to mediate the inhibitory effect of angiostatin on tube formation. While these angiogenesis inhibitors were repressed, endothelial cell markers were induced (e.g., ESM1).

Microenvironment remodeling and invasion. In agreement with a loss of hepatocyte differentiation (see above), we observed a decreased expression of the epithelial marker E-cadherin (CDH1) in more than 70% datasets (Supplementary Table S2). Loss of E-cadherin is frequently observed in cancer and notably contributes to tumor progression by increasing proliferation and invasion. The 935-gene HCC signature was also significantly enriched in genes encoding extracellular matrix proteins (e.g., COLAA1, COLA2, LAMC1). Induction of these genes frequently correlates with changes in the cellular microenvironment associated with liver fibrosis and tumor invasion, a process largely controlled by TGFβ pathway. Interestingly, SMAD2 that mediates TGFβ signals was either induced (80% datasets) or repressed, highlighting the functional duality of the TGFβ pathway, acting as a tumor-promoting or a tumor-suppressing factor in cancer, including HCC (17). SFRP1, acting as a negative modulator of the Wnt/β-catenin pathway frequently activated in HCC microenvironment, was repressed.

Avoiding immune destruction. Several immune-associated genes were identified and most of them were repressed (Supplementary Table S2), including activation markers of cytotoxic T lymphocytes and natural killer (NK) cells (e.g., CD69, KLRIK1, GZMA). In addition, immunoregulatory mediators with chemotactic activity for competent immune cells were repressed (e.g., CCL19, CCL2/MCP1, CXCL12/SDF1).

Emerging hallmarks enriched in HCC. In addition to the well-characterized hallmarks of cancer cells described above, 2 more discrete but promising functional categories emerged from the analysis of the 935-gene HCC signature. These specific hallmarks were associated with protein turnover and epigenetics (Supplementary Table S2). In agreement with an active metabolic activity, we observed an increased expression of several translation-
associated factors, including genes encoding ribosomal subunits (e.g., RPS5, RPL38) and translational machinery (e.g., EIF4G2). Unexpectedly, genes involved in protein ubiquitination (e.g., UBE2A, UBE2C) and degradation through the 26S proteasome pathway (e.g., PSMA1, PSMA6, PSMD2, PSMD4) were similarly overexpressed, evocative of a proteotoxic stress associated with a protein hyperproduction and/or misfolding (18). The second prominent promising HCC hallmark is the upregulation of numerous genes acting at the epigenetic level (Supplementary Table S2). Thus, the 935-gene HCC signature included important regulators of chromatin assembly and remodeling (e.g., CHAF1A, HDAC1, HDAC5, HMGB2), components of the polycomb-repressive complex 2 (e.g., EZH2, SUZ12) that catalyzes the trimethylation of H3K27 (H3K27me3), and master regulators of microRNA processing (e.g., DROSHA).

The 935-gene HCC signature highlights drug candidates for systemic therapies

Next, the cMap algorithm was used to identify relevant drugs in HCC, as previously described (10). We selected drugs that generated gene expression profiles inversely correlated with the 935-gene HCC signature with the highest confidence ($P < 0.003$). Six candidate molecules were identified, including two histone deacetylase (HDAC) inhibitors (trichostatin A and vorinostat), PI3K inhibitor LY294002, mTOR inhibitor sirolimus (also known as rapamycin), $\alpha$-estradiol, and resveratrol (Fig. 2A; Supplementary Table S6). The impact of these drugs as compared with sorafenib that is currently used for the treatment of advanced HCC (19) was evaluated on the viability of 6 HCC-derived cell lines. Remarkably, except for $\alpha$-estradiol, all the drugs significantly ($P < 0.05$) reduced cell viability (Fig. 2B). The SNU-423 cell line was
subtypes highlighted specific mutations (Fig. 3). The classification into molecular subtypes highlighted specific pathways for drug targeting, including TGFβ, WNT, and AKT inhibitors. Conceptually, our study is slightly different, as it hypothesizes that optimized treatments should take into consideration not only the molecular alterations that occur in specific HCC subtypes but also those recurrently altered in all tumors (Fig. 3). However, the definition of core transcriptional hallmarks in HCC has never been really explored in details, so far. Our study fills this gap and demonstrates the existence of a substantial transcriptional homogeneity in HCC. Hence, we provide a robust, exhaustive, and comprehensive signature of 935 genes commonly deregulated in human HCC from data generated in various laboratories and covering all the known etiologies. We hypothesized that analyzing multiple datasets with the same algorithms significantly increases the accuracy of the findings as compared with conclusions raised from single studies. Consistent with the specific objectives of Hoshida's study (i.e., the definition of signatures for molecular HCC subtypes) and our study (i.e., the definition of a common transcriptional fingerprint for all HCC), there was almost no overlap between the genes included in the S1–S3 signatures and our core HCC signature. Indeed, in the 935-gene HCC signature, only 3% 2%, and 13% genes overlapped with the S1, S2, and S3 signatures, respectively (Supplementary Fig. S2). Besides, most of genes overlapping with the S3 signature were metabolism-associated genes that were shown to be relatively upregulated in S3-HCC, as compared with poorly differentiated HCC of S1 and S2 subtypes (5, 6). However, regardless of HCC subtypes, these genes were commonly repressed when compared with the surrounding nontumor tissues, as observed in our core HCC signature. Each of the signatures (i.e., S1–S3 and core) are then specific in term of constituting genes but could be applied to large HCC cohorts. Altogether, we believe that our signature constitutes a unique and specific fingerprint of recurrent and common transcriptional alterations in HCC.

One striking observation is that the 935-gene HCC signature covers almost all hallmark capabilities of cancer cells described previously (Fig. 3; ref. 15). It is noteworthy that none of the genes included in the 935-gene HCC signature were directly related to replicative immortality. This cancer hallmark is largely controlled by telomerase activity. Actually, telomerase has been shown to be reactivated in more than 90% HCC, mostly due to TERT amplification and somatic mutations or HBV insertion in the TERT promoter (3). More than being only recurrent in HCC, genetic

Discussion

HCC is a deadly cancer worldwide, mainly due to late diagnosis and the absence of effective treatments for advanced stages of the disease. A strategy of HCC stratification into molecular subgroups has been developed worldwide with the objective to translate this knowledge into individualized treatments (6, 20, 21). Thus, Hoshida and colleagues reported the existence of 3 major HCC subtypes referred to as S1, S2, and S3 (6). These subtypes were associated with specific biologic and clinical features (Fig. 3). S1 and S2 subtypes included aggressive HCC and were associated with an aberrant activation of the WNT signaling pathway by TGFβ (S1 subtype) or a progenitor-like phenotype associated with MYC and AKT activation (S2 subtype). S3 subtype included good prognosis HCC that exhibited a hepatocyte-like phenotype and CTNNB1 mutations (Fig. 3). The classification into molecular subtypes highlighted specific pathways for drug targeting, including TGFβ, WNT, and AKT inhibitors. Conceptually, our study is slightly different, as it hypothesizes that optimized treatments should take into consideration not only the molecular alterations that occur in specific HCC subtypes but also those recurrently altered in all tumors (Fig. 3). However, the definition of core transcriptional hallmarks in HCC has never been really explored in details, so far. Our study fills this gap and demonstrates the existence of a substantial transcriptional homogeneity in HCC. Hence, we provide a robust, exhaustive, and comprehensive signature of 935 genes commonly deregulated in human HCC from data generated in various laboratories and covering all the known etiologies. We hypothesized that analyzing multiple datasets with the same algorithms significantly increases the accuracy of the findings as compared with conclusions raised from single studies. Consistent with the specific objectives of Hoshida’s study (i.e., the definition of signatures for molecular HCC subtypes) and our study (i.e., the definition of a common transcriptional fingerprint for all HCC), there was almost no overlap between the genes included in the S1–S3 signatures and our core HCC signature. Indeed, in the 935-gene HCC signature, only 3% 2%, and 13% genes overlapped with the S1, S2, and S3 signatures, respectively (Supplementary Fig. S2). Besides, most of genes overlapping with the S3 signature were metabolism-associated genes that were shown to be relatively upregulated in S3-HCC, as compared with poorly differentiated HCC of S1 and S2 subtypes (5, 6). However, regardless of HCC subtypes, these genes were commonly repressed when compared with the surrounding nontumor tissues, as observed in our core HCC signature. Each of the signatures (i.e., S1–S3 and core) are then specific in term of constituting genes but could be applied to large HCC cohorts. Altogether, we believe that our signature constitutes a unique and specific fingerprint of recurrent and common transcriptional alterations in HCC. One striking observation is that the 935-gene HCC signature covers almost all hallmark capabilities of cancer cells described previously (Fig. 3; ref. 15). It is noteworthy that none of the genes included in the 935-gene HCC signature were directly related to replicative immortality. This cancer hallmark is largely controlled by telomerase activity. Actually, telomerase has been shown to be reactivated in more than 90% HCC, mostly due to TERT amplification and somatic mutations or HBV insertion in the TERT promoter (3). More than being only recurrent in HCC, genetic
alterations in TERT represent early events in human hepatocarcinogenesis (22). Importantly, we show that the 935-gene HCC signature was associated with early chromosomal alterations described in human hepatocarcinogenesis (14). Thus, the signature should include relevant candidate biomarkers for early HCC diagnosis, including secreted biomarkers (e.g., SPINK1). In addition, identifying cell surface markers (e.g., ligands and/or receptors) overexpressed in most HCC may lead to new drug targets with high specificity. Accordingly, innovative nanoparticles may be formulated including specific peptides derived from the 935-gene HCC signature, to increase drug delivery while reducing drug side effects. Interestingly, the 935-gene HCC signature highlighted specific hallmarks associated with protein turnover (i.e., synthesis and proteasomal degradation) and epigenetics (Fig. 3). Over-activation of the ubiquitin–proteasome system has been reported in several cancers and proteasome inhibitors, for example, bortezomib provided promising results in treating hematologic malignancies (23). In experimental models of HCC, the combination of sorafenib and bortezomib demonstrated synergistic antitumor effects through AKT inactivation (24).

Through cMap algorithm, drug candidates were identified and validated in several HCC cell lines, including resveratrol, HDAC inhibitors (HDACi), and PI3K/AKT/mTOR inhibitors, in agreement with the landscape of genetic alterations frequently observed in HCC (2). It is noteworthy that cMap results were mainly derived from MCF7 breast cancer cells (Supplementary Table S6) that may harbor a different spectrum of mutations than HCC cell lines. Interestingly, the comparison of genetic profiles though the Cancer Cell Line Encyclopedia project (www.broad-institute.org/ccle) identified a mutation signature consisting in 16 genes mutated both in MCF7 and in at least 3 of 6 HCC cell lines investigated. This signature included key cancer genes involved in EMT, tumor growth, metastasis, and angiogenesis (e.g., AAK1, CDK11B, ILK, MAP3K1, MAP3K14, NCA111, PRKDC, and VEGFC). One can hypothesize that the efficacy of the identified drugs may be related to these signaling pathways. Resveratrol and HDACi were shown to target multiple cancer hallmarks in preclinical models, including effects on growth inhibition, cell differentiation, angiogenesis, and immunosurveillance (25, 26). At the molecular level, resveratrol was reported to modulate cancer-related signaling pathways, including FAS/FASL, PI3K/ AKT, NF-κB, and WNT (26). HDACi-induced growth arrest has been linked to the induction of the CDK inhibitors p21 and p27. HDACi-induced cell death involves caspase-dependent and -independent pathways. HDACi also cause hyperacetylation and inactivation of HSP90, leading to the degradation of proteins that require the chaperone function of HSP90, including some oncoproteins (25). HDACi can block tumor angiogenesis by inhibiting hypoxia-inducible factors and expression of VEGF (27) and impair immunosurveillance by reducing viability and effector functions of NK cells (28). In liver cancer, we showed that HDACi could interfere with tumor–stroma crosstalk (10) and loss of hepatocyte differentiation (29). In HCC, the results of a multicenter phase I/II study in patients with unresectable tumors demonstrated that HDAC inhibition with belinostat was well-tolerated and associated with tumor stabilization (30).

However, the picture is obviously not so simple given that besides sorafenib, most monotherapies evaluated in phase III clinical trials failed to improve the survival of patients with advanced HCC (31). In addition, recent results of combined therapies, for example, sorafenib associated with erlotinib or doxorubicin failed to demonstrate meaningful clinical benefits (32, 33). This raises questions about the optimal backbone not only for drug combinations (31, 32) but also for combined treatment modalities, including surgery with adjuvant multidrug chemotherapies and biotherapies, personalized radioembolization, and immune-based therapies. Our comprehensive 935-gene HCC signature may help resolve this issue by identifying combinations of treatments to target various signaling pathways altered in HCC. We believe that evaluating these strategies in combination with molecules targeting pathways deregulated in specific HCC subtypes may represent promising approaches, particularly in clinical trials where patients for each subtypes are selected on the basis of the expression-specific biomarkers.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: C. Couloumar
Development of methodology: C. Allain, C. Couloumar
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Allain, C. Couloumar
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Couloumar
Writing, review, and/or revision of the manuscript: B. Clément, C. Couloumar
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C. Allain, G. Angenard, B. Clément
Study supervision: C. Couloumar

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