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Molecular Classification of Hepatocellular Carcinoma and Its Impact on Prognostic Prediction and Personized Therapy

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Hepatocellular carcinoma (HCC) is the sixth most common cancer and second leading cause of cancer-related death in the world.[1] The aggressive but not always predictable pattern of HCC causes the limited treatment option and poorer outcome. Many researches had already proven the heterogeneity of HCC is one of the major challenges for treatment option and prognosis prediction. Molecular subtyping of HCC and selection of patient based on molecular profile can provide the optimization in the treatment and prognosis prediction. In this review, we have tried to summarize the molecular classification of HCC proposed by different valuable researches presented in the logistic way.

Key Words: Molecular classification, hepatocellular carcinoma, prognosis prediction, targeted therapy

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer and second leading cause of cancer-related death in the world.[1] It is epithelial in origin, and the tremendous improvement has been made in knowing its transition course and the causative pathogenesis. Majority of the HCC develops in the setting of causative agent such as viral hepatitis, alcohol, etc. followed by fibrosis, cirrhosis with dysplastic changes to invasive hepatocellular carcinoma.[2-4] But, in small population HCC is developed in non-cirrhotic background as in some cases of hepatitis B virus (HBV) infection promoting codon 249 of the tumor suppressor p53 gene mutation, hepatoadenomas, or in post aflatoxin exposure patient.[5] The prognostic information and treatment options provided by clinical and pathological tools achieved certain goals but some questions are still needed to be resolved.[6-8] Currently, the estimation of prognosis and selection of treatment option can be determined by well-known clinical classification system, like Barcelona clinic liver cancer classification[9], but there is no clear marker to distinguish early stage HCC from intermediate or from advanced stage. The same problem does exist in other types of clinical classification as well. The lack of knowledge on HCC initiation in early stage and heterogeneity in intermediate and advanced stage progression is the main existing problem.[10] Therefore, the appropriate classification for grouping patients homogenously improves the treatment modalities and prognostic information. The implication of molecular characterization of HCC is the promising way to overcome this existing problem. Our purpose is summarization of the recent advances in molecular classification of HCC and its impact on prognosis prediction and therapeutic approach.

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Evolution of Molecular Classification of HCC

The recognition of tumor suppressor and promoter activity was the beginning of HCC classification era. Apart from chromosomal aberration, gene mutation is most commonly seen in HCC, which results in oncogene activation and/or tumor suppressor gene inactivation. The most frequently seen tumor suppressor genes are tumor protein p53[11], Retinoblastoma 1 (RB1)[12], cyclin-dependent kinase inhibitor 2A (CDKN2A also known as p16)[13], insulin-like growth factor 2 receptor (IGF2R)[14] and phosphate and tensin homolog (PTEN)[15] while most frequent oncogenic mutations are Beta-catenin 1 (CTNNB1)[16], Axin1 (AXIN1)[17], phosphoinositide-3-kinase (PIK3CA)[18] and V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS2).[19] The early recognition of mutation of these genes in hepatocarcinogenesis brought forward the concept of targeted therapy, which led to development of drugs like PI3 kinase inhibitor. As these genes weren't sufficient

for both the prediction of prognosis and therapeutic implication, therefore persistent research on molecular classification is required. Many researches were performed to obtain the expression data of HCC and proposed different classification systems,[20-30] some of them were deal with differentiation of the pathogenesis of HCC, identification of HCC nature, prognosis prediction, and potential targeted therapy, classification systems correlated the pathophysiologic phenotype, while some researchers were focused on molecular expression irrespective of the other variables.

Gene Profiling of HCC on Pathogenic Basis

In 2001, Okabe et al. reported the result of genome-wide analysis of gene expression of HCC using cDNA microarrays in 20 primary HCCs with the corresponding noncancerous tissues showing distinct pattern of gene expression between HBV and HCV induced HCC.[21] They found out that phase I modification enzymes of carcinogen (CYP2E, AKR1C4, EPHX1, FMO3) were upregulated in HCV-related HCC, while phase II conjugation enzymes of carcinogen (UGT1A1, UGT2B10, GPX2) were downregulated in HBV-related HCC, which helped further understanding in development and progression of HCC with each pathogenic background. This work was elaborated by Iizuka et al. further to explore the distinguishing molecular characteristics between HBV and HCV associated HCC and revealed 83 genes whose expression markedly differed in these two types of HCC.[26] The research team pointed out 31 of 83 genes (imprinted genes and genes related to signal transduction, transcription and metastasis) were upregulated in HBV associated HCC while remaining 52 genes (genes responsible for detoxification and immune response) were upregulated in HCC with HCV background which favored the result of okabe et al.

Recently, new concept of deriving molecular subtyping of HCC such as microanalysis of demographical etiology in molecular level has been put forwarded. Lately, according to an article, 348 HCC and 375 chronic liver disease in Indian population were studied and showed that HBV is the predominant etiology of HCC followed by HCV and genotyping revealed that HBV genotype D (odds ratio, OR=1.76) and mixed genotype (A+D, OR=6.86) had higher risk of HCC development whereas no significant differentiation was observed among the HCV genotypes on HCC development.[31] In addition, high HBV DNA was associated with the increased risk for development of HCC (OR=2.26) while HCV viral load didn't correlate with the risk of HCC development. Moreover, they observed distinct clinical characteristics among the HBV genotypes, however, there were no significant differences in clinical features were observed in cases of HCV genotypes. This work was further supported by the study of Ringelhan et al. which was study that they summarized the oncogenic effects of HBV as integration in the host genome causing the deletion, translocation, cis/trans activation, production of fusion transcript and genomic instability.[32] Further, a newly finding suggested that aberration of tumor suppressor properties of interferon regulatory factor 2 (IRF2) due to its inactivation was predominantly seen in HBV associated HCC.[33] This microanalysis of etiologic factor could be identified the high-risk patient for developing HCC and their integration in molecular classification help in the development of novel therapeutic approaches.

Heterogeneity in Metastatic and Non-metastatic HCC

Previously, the prediction of recurrence and prognosis of HCC patients were based on clinicopathologic features such as tumor size, pathologic grade of tumor, age, liver function, portal vein thrombosis, microvascular invasion, etc.[34-37] However, the prognostic significances and liability in clinical application wasn't satisfactory.[8] To overcome this, Iizuka et al. carried out oligonucleotide microarray analysis for the prediction of early intrahepatic recurrence of HCC after curative surgery experimented in 33 tumors and verified in 27 new patients that was published in 2003.[38] They predefined early intrahepatic recurrence as the recurrence within one year after curative surgery and formulated the predictive system consisting of 12 genes to classify HCC into early intrahepatic recurrence or non-recurrence with 93% accuracy, 88% positive predictive value and 95% negative predictive value, but verification in larger sample was needed. Kurokawa et al. carried out further research on molecular prediction of early intrahepatic recurrence in larger sample based on gene expression using polymerase chain reaction (PCR) array in 2004.[39] They carried out experiment in 100 HCC samples and pointed out the differential expression of 92 genes between early intrahepatic recurrence and non-recurrence and showed the comparable result when predicting with 20-top ranked genes with the accuracy rate of 72.5% that was verified in 60 samples.

In 2003, we applied cDNA microarray based gene expression profiling and revealed that intrahepatic metastatic lesions are identical to their corresponding primary tumor but dis-

tinguishable features were present in between primary metastasis-free HCC and primary HCC with accompanying intrahepatic metastasis which can be differentiated by 153 relevant genes and could predict intrahepatic metastasis and prognosis with an overall accuracy of 78%.[40] We (Roessler et al.) further analyzed the predictability of these 153 metastatic genes in 2 independent cohorts consisting of total of 386 patients who received radical resection and verified that gene signature was predictive of overall and disease free survival, especially early disease (small (<5 cm) and solitary tumors), independently of clinical characteristics and microarray platform. The metastatic gene signature predicted overall survival (OS) and disease-free survival (DFS) more accurately in early recurrence (<2 years) cases.[41] Furthermore, we (Budhu et al.) investigated the role of microenvironment in venous metastasis, recurrence and prognosis in HCC where we compared the gene expression profile of 115 noncancerous surrounding hepatic tissues using cDNA and constructed a refined expression signature of 17 genes that were determined by quantitative real time polymerase chain reaction (qRT-PCR), which could successfully predict the venous and extrahepatic metastasis with overall accuracy of >92%.[42] The study also showed that these genes were significantly associated with the prognosis. Interestingly, our team found the tumor metastasis was associated with the inflammatory modulation where metastatic tumor group showed increase in Th2 cytokines and decrease in Th1 cytokines and verified that Th1-Th2 cytokine profile was regulated by macrophage colony stimulating factor1 (CSF1). However, the predictive genes among these studies, 153 genes listed by Ye et al. and 17 genes for the prediction of venous metastasis by the same research team, 12 genes sorted out by Iizuka et al., and 20-top ranked genes pointed out by Kurokawa et al. weren't overlapped.

It is worth to mention other important study in the field of molecular classification of HCC that was reported by Chen et al. in 2002 showing the gene expression pattern in normal liver tissues, benign tumors, primary HCC, and metastatic HCC.[30] This study showed the recognizable gene expression pattern among these different samples, which was in consistent with previous result, and also further verified the heterogeneity among the different tumors of same pathological type, even in the same patient. Researchers identified 91 genes expression profile, which could distinguish between invasive and non-invasive hepatocellular carcinoma. Furthermore, they showed the distinct gene expression pattern between primary and metastatic liver cancer.

Clinical Outcome Prediction by Molecular Profiling of HCC

Several studies were focused on prognosis of HCC patients through molecular characterization by different study designs. In 2004, one of the major studies was published by Lee et al., showed that molecular classification of HCC could predict the survival of patients.[43] They analyzed gene expression profiles of 91 human HCCs using DNA microarrays and identified two distinct groups (cluster A and cluster B) of HCC associated with survival and validated by 5 independent supervised learning methods. The two subgroups identified by distinct molecular features showed obviously different length of survival. The characteristics of each subgroup are listed in table (Table 1). Further work of Lee et al. on molecular classification of HCC in 2006, which was investigated

Table 1. Characteristics of molecular subtypes of HCC proposed by Lee et al. and Hoshida et al.

Author	Subtypes	Prevalence	Characteristics
Lee et al.[43]	Cluster A (Lower survival)	44%	1. OS (30.03 ± 8.02) months 2. Overexpression of HIF1A 3. Higher expression of cell proliferative markers such as PNCA, CDK4, CCNB1, CCNA2 and CKS2 4. Higher expression of anti-apoptotic, ubiquitination and sumoylation genes
	Cluster B (Higher survival)	66%	1. OS (83.7 ± 10.3) months 2. Lower expression of cell proliferative markers 3. Lower expression of anti-apoptotic, ubiquitination and sumoylation genes.
Hoshida et al.[52]	Good prognosis	65% (62% in longer follow up patient)	Genes associated with normal liver function: plasma proteins and drug metabolizing enzymes (ADH5, ALDH6, AKR1A1, AKR1D1, ALDH9A1)
	Poor prognosis	35% (38% in longer follow up patient)	Genes associated with the inflammation (interferon signaling, activation of NF-κβ and TNFα signaling)

HIF1A, hypoxia inducible factor 1A; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; AKR, aldo-keto-reductase; NF-κβ, nuclear factor-κβ; TNFα, tissue necrosis factor α

on 139 patients based on 941 genes and classified tumor cell types into hepatocyte-like or fetal hepatoblast-like genotype showing strong correlation with survival prognosis.[44]

In 2007, Wang et al. reported gene profiling of HCC related to clinical outcome to identify gene signatures determining HCC recurrence.[45] They verified the presence of both vascular invasion and cirrhosis at the time of diagnosis was sufficient to predict early recurrence but neither of them alone were insignificant. The importance of their work lied on formulation of 57-gene signature for the prediction of HCC recurrence with the existence of either vascular invasion or cirrhosis at the time of diagnosis showing 84% accuracy, by molecular profiling on the basis of oligonucleotide probe assays using a pool of 23 HCC and verified in 25 HCC patients. Their work further helped to stratify the patient with different risk of recurrence. Not surprisingly, same as in the previous studies, the 57 genes were not identical to any of the gene signature shown by Ye et al., Budhu et al., Iizuka et al. and Kurokawa et al.[38-40,42]

Many studies in gene expression profile have already verified the different gene expression pattern in different stages and different etiologic factors.[21,26,30,43,46,44,47] Considering the specificity of etiological factor in molecular characterization of HCC, Woo et al. observed the gene expression profile of 65 HCC patients with HBV infection and generated 628 gene features which can stratify HCC patients into high risk and low risk recurrence (cut off point was 1 year after surgery) with the prediction accuracy between 83% and 97%.[48] Their further analysis showed CD24 as the most valuable biomarker for the prediction of early recurrence that was regulated by auxillary factors Sp1 and peroxisome proliferator-activated receptor-α.

The correlation between the different microarray based aforementioned studies was poor. Discrepancy in accuracy for predicting prognosis might be the differentiation of sample size, the application of different algorithm and distinct point of studies.[49] The lack of consistency and robustness of these predictive markers generated on different microarray analysis from different studies were one of the major drawback in their clinical application.[50,51] With the purpose of overcoming this problem, Hoshida et al. performed gene expression profiling in fixed, not frozen, tissues from 307 patients (82 in training set and 225 in validation set where 168 patients were followed up for longer duration) with HCC and revealed that gene expression profile from tumor tissue didn't significantly correlate with survival prognosis, instead, gene expression profile from nontumoral tissue highly associated with survival prognosis.[52] This was in contrast to the previous studies. According to the prognostic indicator, the gene expression pattern of surrounding tissue stratified patients into good or poor prognosis groups (Table 1). This study also altered our understanding of early recurrence and late recurrence, late recurrence should be also considered as the second primary tumor presumably due to the carcinogenic effect of cirrhosis.[8,53,54] In a multivariate analysis showed that 186 genes from surrounding tumor were the strongest predictor of the late recurrence. We also identified the importance of surrounding tumor tissue, as mentioned previously, with the result showing that immunosuppressive response promoted the intrahepatic metastasis.[42]

A recent study carried out by Nault et al. reported 5-gene score associated with survival to stratify HCC patients.[55] They analyzed the gene expression pattern of 314 HCC samples associated with disease specific survival and identified 5 genes (HN1, RAN, RAMP3, KRT19, and TAF9), which were validated in 125 patients formulating 5-gene score. The 5-gene score was also validated in 2 groups of HCC patients (n=434), which could also predict the overall survival, early tumor recurrence, and ultimately stratify HCC patients into good and poor prognosis groups. These two classification systems also referred the distinct therapeutic strategies between the subgroups, for example, targeting HIF1A in poor survival group proposed by Lee et al.[43] while poor prognosis subgroup of Hoshida et al. can be treated by targeting NF-κβ pathway and other inflammatory cascades.[52]

Integrative Approach for HCC Characterization

In 2004 Kai Breuhahn et al.[46] analyzed the expression of mRNA motif in 43 different human HCC samples and 3 HCC cell lines using high-density cDNA microarrays and revealed 2 distinct subgroups of HCC. The subgroups were related to intratumor inflammation and tumor cell apoptosis. The distinguishing features of each subtype are summarized below(Table 2).

The classification system proposed by Kai Breuhahn et al. also played important role in determining the therapeutic strategy as IGF-II overexpression in B1 subgroup has been associated to reduced apoptosis and increased proliferation which could be inhibited by IFN-γ treatment.[46]

One of the important studies on molecular classification of HCC was the work of Boyault et al.[47] The research performed global transcriptome in 57 HCCs and 3 hepatocellular adenomas and validated in 63 additional HCCs by using

RT-PCR and proposed 6 robust subgroup of HCCs (G1-G6) associated with clinical and genetic characteristics. The distinguishing features of each of the subclass have been listed in the table below (Table 3).

Boyault et al. didn't mention the correlation of the subtypes with the survival prognosis but showed some clinical impor0 tance as almost 50% of the tumors were related to Wnt or Akt activation, which could be the potential targeted molecule in treating HCC of particular subtype.[47] However, they showed molecular subtype G3 was significantly associated with the tumor recurrence, which was also verified by 5-gene score formulated by themselves to improve the prognosis accuracy.[55] Furthermore, the metabolomics profile didn't show any differences in lactate, glucose, glycerol-3-phosphate, alanine, malate, and myoinositol among the subgroups for suggesting equal operation of glycolysis in each tumor subtype instead of elevated Wnt signaling and β-catenin activation in G5 and G6.[56] However, they found tissue concentration reduction of palmitic acid, 1-steroylglycerol and 1-palmitoylglycerol due to the overexpression of lipid catabolic enzymes in G1 subtype.

Chiang et al. reported another novel molecular subtyping of HCV-related HCC from unsupervised classification with consensus hierarchical clustering of 91 tumors and divided into 5 subtypes, naming as (1) CTNNB, (2) proliferation, (3) IFN related, (4) polysomy chromosome 7 and (5) unannoted.[57] The distinguishing features of each of the subtypes are summarized in Table 4. A further step to overcome the previously yielded divergent result and to develop robust molecular classification of HCC, the work proceeded by this research team was reported in 2009.[58] They performed integrative transcriptome meta-analysis of 8 independent patient cohorts across the world with the additional inclusion of 118 formalin fixed, paraffin embedded tissues and analyzed in 603 patients with mainly HBV and HCV associated HCC and divided HCC into three robust subclasses naming as

Table 2. Characteristics of molecular subtypes proposed by Boyault et al.[46]

Subtypes		Prevalence	Characteristics
A group		65%	1. Higher level of immune response genes, IFN-regulated genes (IFI35, IFI27, ISG15) 2. Higher expression of apoptosis related genes (CD40, apoptosis inducing factor) 3. Lower expression of IGF-II 4. Increased apoptosis and infiltration
B group	B1	14%	1. Over expression of IGF-II 2. Not IFN-regulated 3. Low level of apoptotic genes 4. Reduced apoptosis and infiltration
	B2	21%	1. Downregulation of IGF-II 2. Not IFN-regulated 3. Low level of apoptotic genes 4. Reduced apoptosis and infiltration

IFN, Interferon; IFI, IFN induced gene; ISG, IFN-stimulated protein; IGF-II, Insulin like growth factor II

Table 3. Characteristics of molecular subtypes proposed by Boyault et al.[47]

Subtypes	Prevalence	Characteristics
G1	10%	Chromosomal instability; overexpression of cell cycle/ proliferation/ DNA metabolism genes; HBV positive; overexpression of genes encoding for myosine heavy chain IIb and transcription factor SOX9, overexpression of IGF2, IGF1R, PEG3, PEG10 and SGCE; overexpression of AKT; AXIN1 mutation
G2	14%	Chromosomal instability; overexpression of cell cycle/ proliferation/ DNA metabolism genes; HBV positive with high viral DNA copies; enhanced AKT expression, PIK3CA and TP53 mutation;
G3	12%	Chromosomal instability; overexpression of cell cycle/ proliferation/ DNA metabolism genes; HBV negative; overexpression of genes encoding for nucleus import/export proteins; hypermethylation of CDKN2A; TP53 mutation
G4	34%	Chromosomal stability; enriched for TCF1 mutation
G5	20%	Chromosomal stability; hypermethylation of CDH1; β-catenin activation; down regulation of genes involved in the response to biotic stimuli and immune response.
G6	10%	Chromosomal stability; hypermethylation/low expression of CDH1; β-catenin activation

IGF2, Insulin growth factor 2; IGF1R, Insulin growth factor receptor 1; PEG, Paternally expressed gene; SGCE, Sarcoglycan epsilon

Table 4. Characteristics of molecular subtypes proposed by Chiang et al. and Hoshida et al.[57]

Subtypes	Prevalence	Characteristics
CTNNB	26%	1. Enriched for CTNNB1 exon 3 mutation 2. Overexpression of liver-specific marker genes such as GLUL, LGR5, TBX3, REG3A 3. Larger tumor size, diameter >3 cm
Proliferation	25%	1. Overexpression of proliferation related genes and proliferation driven by tyrosine kinase activation 2. Chromosomal instability 3. Higher frequency of 4q loss and 13q loss. 4. Correlation with macrovascular invasion and higher expression of AFP marker
IFN related	20%	1. Overexpression of interferon-stimulated genes: STAT1, ISG15, IF16 and IF127 2. Lower expression of both CTNNB1 exon 3 mutation and IGF-II 3. Smaller tumor size, diameter <3 cm.
Polysomy chromosome 7	10%	1. Polysomy of chromosome 7, >2.7 times of median copy number 2. Overexpression of genes such as COBL, CLDN15, MAD1L1, POLD2 and EPHA1
Unannotated	19%	1. Focal gain of 6p21 2. No any specific markers, further research needed.
S1	Clinical data set (28-31%)[59]	1. Activation of WNT signaling pathway
S2	Clinical data set (23-24%)[59]	1. MYC activation 2. Enriched for EpCAM positivity 3. Enriched in AKT activation 4. Overexpression of AFP 5. Suppression of interferon targeted genes
S3	Clinical data set (45-49%)[59]	1. Activation of p53 and p21 target gene sets 2. Overexpression of APO/ALDH/ADH, CYP, coagulation and oxygen radical scavenging family genes

AFP, alpha-feto protein; APO, apolipoprotein; CYP, cytochrome P450

S1, S2, and S3. The characteristic features of each subtype are listed below (Table 4). Furthermore they correlated the expression data with the clinical presentation.

The characteristics of some of the subgroups proposed by Chiang et al. overlapped with the previously reported molecular subtype of HCC. The CTNNB subtype overlapped with high survival group of Lee et al. and G5 and G6 subtypes of study of Boyault, et al. Similarly, the Proliferation subtype overlapped with low survival subgroup of Lee et al. and G1-G3 subclasses of Boyault et al.[43,47,57] The overexpression of interferon-stimulated genes of IFN subtype were overlapped with A group of Kai Breuhahn et al.[46,57] While, the S1 and S2 subclass proposed by Hoshida et al. shared some common characteristics with poor survival subgroup defined by Lee et al.[43] and Proliferation subgroup mentioned by Chiang et al.[57] S3 subclass overlapped with CTNNB group of Chiang et al.[57] Similar subtypes were proposed earlier by Lee et al. where microarray analysis of human hepatoma cell lines revealed subtypes with one group showing activation of oncofetal promoter leading to increased expression of AFP and IGF-2, while other group showing overexpression of genes associated with invasion and metastasis.[60] The subclasses proposed by Hoshida et al. on the basis of integrative proteome analysis were also relevant to clinicopathologic phenotype. Concretely, S1 subgroup was associated with more vascular invasion and satellite lesions with early recurrence and poor prognosis, while S3 subclass exhibited the majority of good survival gene signature. After thorough investigation of this research team concluded that "early recurrence" associated with malignant characteristics of primary tumor and had less impact in patient survival in earlier stage of HCC, while "late recurrence" was determined by the biological state of surrounding liver at risk.

The continuous work in molecular characterization of HCC by this research team found out marked overexpression of fibroblast growth factor receptor 4 (FGFR4) in S2 subgroup, but not in non-S2 subgroups, which further supported the validation of this classification system.[59] Previously, other researchers have already demonstrated the association of FGF and FGFR signaling with the development of numerous human cancers, which enhances the proliferation of tumor via mitogen-activated protein kinase (MAPK) pathway.[61,62]

Expression of FGFR4 in mature hepatocyte and its role in hepatic carcinogenesis have been already verified.[63-65] The research team concluded that S2 subgroup HCC was sensitive to FGFR tyrosine kinase inhibitors. Byproducts of development of anti-angiogenic drug created the first generation of FGFR inhibitory drug,[66-68] while the second generation with less activity on vascular endothelial growth factor receptor 2 (VEGFR2) is under clinical investigation.[69] The molecular classification proposed by Chiang et al. wasn't associated with prognosis but carried some important information in therapeutic implication as CTNNB group can be treated by targeting to Wnt signaling pathway while in case of proliferation subtype, therapy can be focused on targeting to proliferation related genes.

Gene Profile in Determination of Pathophysiologic Phenotype

In the course of molecular characterization of HCC, Katoh et al. published research article focusing on classification of HCC into genetically homogenous subclasses in 2007.[70] They observed the genome scale chromosomal copy number alteration profiles and mutational statuses of γ-catenin and p53 in 87 HCCs and verified the possibility of dividing heterogeneous HCCs into homogenous subclasses correlating with the clinicopathologic features (c-myc induced HCC, 6p/1q-amplified HCC, 17q-amplified HCC etc.). The research team further proved the possibility of novel targeted therapy that might be useful for particular subtype as rapamycin inhibited the proliferative activity of HCC with 17q amplification.

In the same year, Wurmbach et al. reported the correlation of pathological transition and molecular signature of HCV associated HCC.[71] They performed the gene expression profile of 75 tissue samples dividing into 5 groups on the basis of pathological stages: (1) normal, (2) cirrhosis, (3) dysplasia, (4) early HCC and (5) advanced HCC and revealed the number of genes differentiating between the consecutive stages as 8 genes, 24 genes, 93 genes and 9 genes. They also showed certain genes upregulation and deregulation of pathways characterizing each stage, such as upregulation of one gene (CLDN10) and deregulation of Notch and Toll-like receptor pathways in cirrhotic stage, upregulation of three genes (GREM2, EPO and NRG1) and deregulation of cytokine-cytokine receptor and JAK/STAT pathway in dysplastic stage; upregulation of four genes (ASPM, PRIM1, HMMR and IRAK1) and pathway analysis revealed deregulation of all the 15 tested pathways; and advanced HCC is characterized by upregulation of genes involved in proliferation, DNA repair and replication, ubiquitin cycle etc. Many genes and deregulated pathways mentioned in this research were common as reported by previous researches even though discrepancies in etiologies, sample collection and even in study design were observed.[21,38,40,72-75] The other lately published article by Wood et al. proposed new molecular subtype that they carried out the pathological analysis of 219 HCC and found that a group of HCC specimens showed distinct histological character and distinct molecular features.[76] This particular group was designated as chromophobe hepatocellular carcinoma with abrupt anaplasia, which was associated with alternative lengthening of telomere (ALT) phenotype verifying distinct molecular subtype.

We also verified that molecular profiling of HCC could successively distinguish between pathologic and non-pathologic tissues, which was reported in 2009.[77] we analyzed microRNA (miRNA) expression profiling in a cohort of 241 HCC samples and revealed that pathologic tissue showed reduced level of miR-26 as compared to non-pathologic tissue and also pointed out its association with HCC. Further analysis on miR-26 supported this result as we confirmed the suppressive role of miR-26a in tumor growth, angiogenesis and metastasis.[78,79] This finding was important in the determination of prognosis and therapeutic implication as we showed that low miR-26 expression had shorter survival but better response to interferon therapy. Similarly, a study in classification of HCC was reported lately on the basis of miRNA analysis. Wei et al. performed miRNA analysis in 60 HCC training sets and formulated 30 miRNA signature to distinguish pathologic and non-pathologic tissues and 20 miRNA for the prognostic signature, which then validated in 50 test set and 56 independent cohort.[80] This miRNA signature when tested to classify cancerous and non-cancerous tissue provided the accuracies of 97% and 90% in test set and independent cohort respectively. Likewise, another research performed miRNA analysis in liver core biopsy to distinguish primary tumor from metastatic cancer. Perell et al. performed miRNA analysis in 199 primary resected samples (with 162 primary resected tumors which included 9 classes of primary tumors and 37 normal liver resections) and developed 55 miRNA expression profiles using PRIM classifier, which then tested in 79 liver core biopsies (containing 57 metastatic, 7 primary liver cancer and 15 normal liver samples) with cross validation accuracy 67.1% and validated in 55 liver core biopsies (containing 35 metastatic, 10 primary liver and 10 normal liver samples) with an overall accuracy of 74.5%.[81]

A study was reported in 2010 by Malenstein et al., where the research team formulated 265 genes from microarray experiments with HepG2 liver cells under chronic hypoxia and included previously mentioned three microarray studies (Lee et al.[43], Boyault et al.[47] and Wurmbach et al.[71]) to determine 7 gene set of prognostic value after several selection step and validated in 91 patients (from Chiang et al.[57]).[82] They found that 4 (CCNG2, EGLN3, ERO1L and WDR45L) of the 7 genes were upregulated and remaining 3 (FGF21, MAT1A and RCL1) were downregulated under hypoxia and derived the gene scoring system where median survival of patients with a score of >0.35 was 307 days, while median survival of patients with score of <0.35 showed 1,602 days, stratifying HCC patients into high hypoxia score and low hypoxia score with clearly distinct prognosis.

CONCLUSION

One of the biggest challenges in treating HCC patient is phenotypic and molecular diversity, which arose the concept of personalized treatment. Molecular characterization of HCC is the preconditioned requirement to initiate personalized treatment approach. The advancement in genomic technique improved the determination of molecular profile of HCC and further helped in evaluation of deregulated pathways. Molecular profiling can provide the sufficient information on mechanism of cancer development and acts as powerful biomarkers for the prediction of prognosis and implication of therapy. HCC has been well known in clinical field for decades but the treatment outcome still remains poor, this might be due to disease based therapeutic approach in the earlier eras and the result didn't meet our expectation, therefore, patient targeted therapeutic approach is the ultimate requirement. Currently, sorafenib, a multikinase inhibitor that targets the serine threonine kinases Raf-1 and BRAF, vascular endothelial growth factor (VEGF) and platelet derived growth factor B (PDGFB), is the only drug approved by FDA for the treatment of HCC since 2008, after the result of sorafenib hepatocellular carcinoma assessment randomized protocol (SHARP) phase 3 clinical trial showing 3 months prolonged median survival.[83] To overcome so-called 10-month anti-angiogenic ceiling effect of sorafenib in HCC, various drugs as first line and second line were experimented in clinical trials, however, none showed superior to sorafenib.[84,85] Therefore, continuous research on molecular basis for better molecular profiling of HCC is needed which ultimately improves the treatment outcome. A plethora of genomic, transcriptomic, proteomic and metabolomics investigation yielded molecules that are both up- and down-regulated, however, no real consensus has emerged regarding exploitable biomarkers for the prediction of prognosis and therapeutic implication in HCC. Future study should be designed considering the drawbacks of past studies to improve the molecular profiling of HCC and better understanding the molecular mechanisms, which ultimately helps to achieve our goal to treat HCC successfully.

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