Translational research for hepatocellular carcinoma: What's new?

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Abstract

Hepatocellular carcinoma (HCC) is a heterogeneous disease that usually develops within liver cirrhosis. Cancerogenesis in HCC is not a clear process and, at present, there is not a well-defined sequence of DNA mutations able to explain the entire process, from normal hepatocyte to HCC. Lately, the impact of some oncogenes on HCC development has been studied and some of these genes belong to the MAP-Kinases pathway, highlighting the importance of downstream effectors stimulated by the interaction between extracellular growth factors and tyrosine kinase receptors. Unfortunately, drugs able to interfere with the aforementioned pathway showed no positive results in clinical trials. A number of preclinical studies have focused the attention on epigenetic changes in HCC cells, focusing on the extensive DNA hypermetilation as a factor causing the knockout of several tumor suppressor genes. Cancerogenesis of HCC, at least at an early phase, could be sustained by epigenetic changes. Finally, some authors have tried to classify HCC on the basis of gene mutations found after performing an extensive genome sequencing, and interestingly, they have identified different classes of HCC on the basis of different clusters of mutated genes. HCC is not characterized by a unique driver mutation, as the case of EGFR mutations for lung cancer or K-Ras mutations for colorectal cancer, thus it is, at present, very difficult to identify a reliable target for antitumoral therapy. This review focuses on current translational research and molecular targets in the treatment of HCC.

Introduction

Hepatocellular carcinoma (HCC) is a heterogeneous malignancy of the liver and occurs predominantly in patients with underlying chronic liver disease and cirrhosis, primarily associated to Hepatitis B and C virus (HBV/HCV) infection.[1] During cirrhosis, hepatocarcinogenesis becomes a multi-step process where pre-cancerous dysplastic macronodules transform into early HCC that progress into *small and progressed HCC*, and finally in advanced HCC. Moreover, HCC can develop directly in normal liver caused by malignant transformation of hepatocellular adenomas.[2] Due to this *chameleonic* activity, HCC lacks of effective therapies with poor objective response and overall survival for the few available drugs. Many trials with targeted therapy failed during the last 20 years[3] and several factors are responsible for this failure: excessive drug toxicity (especially in cirrhotic patients with poor liver function), lack of significant antitumoral potency, poor understanding of mechanisms responsible for tumor progression, and absence of predictive biomarkers of response.[4]

For these reasons, unraveling the patterns of genomic alterations in these heterogeneous tumors is pivotal towards identifying targeted and personalized treatment for HCC. Therefore, translational research has started with the aim to improve genomic characterization of HCC. In the last years, following and in line with promising results of translational research in several other cancers, HCC treatment made important progress.

Gene changes in hepatocellular carcinoma

In HCC, several data confirmed that differences exist not only among patients, but also between different tumor nodules in the same liver. Cancer stem cells, a subset of cells bearing stem cell characteristics indispensable for tumor development and perpetuation, play a pivotal role in this intrinsic heterogeneity.[5] Several preclinical data suggested that HCC with high stem cells fraction became highly aggressive with early metastatic potential.[6] Based on these data, a recent genomic-based analysis, in patients bearing HCC, was performed and two different subclasses of HCC were identified, on the basis of molecular characteristics and proliferative and non-proliferative genotypes (activation of RAS, mTOR, and/or IGF signaling, for example). A first subgroup was characterized by Wnt/TGF-β pathway deregulation. The other subgroup named *progenitor cells type*, showed to express epithelial cell adhesion molecule and cytoskeletal activation markers. This last was associated with a major probability that HCC is diagnosed in *early stage*.[7] The aforementioned acquisition has paved the way to translational researches in HCC.

Recently some studies proposed genome sequencing with the aim to identify genes able to drive cancerogenesis in HCC, and thus, analyzing their therapeutic implications. Schulze *et al*. evaluated whole exome sequencing of HCC with the aim to identify actionable mutations, suitable to be therapeutic targets. They identified 161 putative driver genes associated with 11 pathways of recurrence. Three main groups of genes were identified, one of them (belonging to CTNNB1 family, beta catenin) correlated with alcohol consumption, another (belonging to TP53 pathway) correlated with HBV infection, and the last (AXIN1 family) showed no correlation with any risk factors.[8]

Analyses according to tumor stage progression, revealed TERT (telomerase reverse transcriptase) promoter mutation as an early event, whereas FGF3, FGF4, FGF19/CCND1 amplification, TP53 and CDKN2A alterations, appeared at more advanced stages and in aggressive tumors. These genetic alterations occurred in 28% of HCC, and they are potentially targetable

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by drugs, some of which are already FDA-approved.[9]

Authors concluded that these risk factor-related specific mutational signatures could be useful to design clinical trials for targeted therapy in HCC.[10]

Another potential therapeutic target is the Telomerase. Activation of Telomerase is the earliest and most frequent alteration in the HCC cancerogenesis, and mutations in TERT promoter occurred in 60% of cases.[11]

TP53 and *CTNNB1* gene mutations, other than gene alteration involving the *Wnt* pathway, are often involved in the process of cancerogenesis, so they can represent other potential therapeutic targets for therapy against HCC.[11]

Different carcinogenic signaling key-pathways could be examined in HCC cells, with the aim to identify selective inhibition activity against cancer cell growth. Unfortunately, in HCC several oncogene mutations have not foreseeable consequences.[12] An example is Wnt, that plays a pivotal role in other cancer types evolution, but in HCC there is not a direct correlation with cancer development. Another disappointing case, despite a strong rationale identifiable in preclinical models, is the RAS/MAPK (mitogen activated kinase) pathway, present in the majority of HCC subtypes. Albeit PI3/AKT/mTOR (mammalian target of the rapamycin) and MAPK pathways are notoriously related to cell proliferation and apoptosis and survival in solid tumors, lots of biologic drugs targeting MAPK pathway failed to improve overall survival in HCC patients, suggesting that other pathways are probably involved in HCC cell proliferation.[13]

Immune system in hepatocellular carcinoma

Interestingly, some interleukins are possible targets for studies in HCC. Improving immune cells function with cytokine therapy may be effective therapeutic strategy in HCC treatment. In the real practice, several HCC patients suffer for early recurrence after liver resection and loco-regional therapy (*i.e.* radiofrequency). Fu *et al* demonstrated that overexpression of interleukin-35 (IL-35) is associated with HCC aggressiveness and recurrence after curative resection.[14]

The definition of HCC as a *chameleon* and heterogeneous disease is confirmed by another recent study carried out by Long *et al*. Contrarily to what showed by Fu *et al*., the authors demonstrated that IL-35 expression was significantly lower in patients with advanced stage HCC, if compared with those at early stage. IL-35 over-expression in HepG2 cells significantly upregulated HLA-ABC and CD95, reduced activities of MMP-2 and MMP-9, and decreased cell migration, invasion and colony formation capacities.[15] Despite these conflicting data, IL-35 remains a potential and promising study target in HCC.

Epigenetics and hepatocellular carcinoma

Aberrant DNA methylation in HCC represents another promising field to investigate. Aberrant DNA methylation leads to altered gene expression, resulting in cancerous features. Lack of common genetic markers associated with HCC (*i.e.* p53, AXIN 1) strongly suggests that these epigenetic alterations could be the predominant alternative factor contributing towards liver carcinogenesis.[16]

DNA methylation occurs when a methyl group is attached to the fifth carbon of the cytosine nucleotide and this process is catalyzed by DNA methyltransferases. Hypomethylation affects the structural-nuclear function by promoting chromosomal and genomic instability, while hypermethylation is often associated with the silencing of tumor suppressor genes. During early-stage of hepato-carcinogenesis, aberrant DNA methylation on chromosome 16 (D17S5 locus) and chromosome 3 occurred, with inactivation of multiple tumor suppressor genes.[17] Probably, in the post-initiation phase (promotion-phase), tumor progression is driven primarily by epigenetic alterations induced by carcinogens, including increased histone H3 lysine 9 and histone H3 lysine 27 trimethylation of the tumor suppressor genes RASSF1A (Ras association domain-containing protein 1), p16 (INK4a), suppressor of cytokine signaling (SOCS)1, E-cadherin 1 (CDH1) and Cx26, and early RASSF1A. These changes determine dysregulation of the balance between cell proliferation and apoptosis, a fundamental protumorigenic event in hepatocarcinogenesis.[18]

A common tumor suppressor involved in epigenetic modification through methylation and repression of the promoter region is p16. Different studies showed that the impact of p16-disruption on carcinogenesis is strongly variable in different geographic regions. Evidently, environmental factors may affect the frequency and concordance of the degree of hypermethylation. DNA methylation may potentially predict the risk of tumor development, tumor staging, patient survival and HCC recurrence. In order to translate these markers into actual clinical use, prospective studies and validation methods are required.

Another potential therapeutic field comes from the *extensive world* of micro-RNA blockade strategy. Micro-RNA (miRNA) are a class of short non-coding RNAs that control the translation of targeted messenger RNAs through complementary interactions with the 3' untranslated region of target genes, and they are involved in several cellular processes, such as embryonic development, cell cycle progression, metabolism and also carcinogenesis. Several studies have shown that various miRNAs are deregulated (up- or down-regulated) in human HCC.[19] Expression of some miRNAs correlated with different

Table 1. Possible bimolecular targets in hepatocellular carcinoma.

Genetic or epigenetic aberration in HCC	Drugs potentially able to target it
TP53 mutations	Virus able to attack P53 deficient cells (ONYX015)
Axin1 mutations	Drugs able to reactivate of p53 and induction of tumor cell apoptosis (RITA)[23,24]
CDKN2A mutations	Drugs able to block the function of heat shock protein 90 (Geldanamycin)[25,26]
Wnt mutations	TERT inhibitors (Imetelstat)[27,28]
TERT mutations	Methyl-transferase inhibitors (5azacitidine)[29-31]
CTNB1 mutations	
INK4 hyper-methylation	
RASSF1A hyper-methylstion	

HCC, hepatocellular carcinoma.

pathogenic agents involved in HCC promotion, such as HCV (*i.e.* miR-196), HBV infection (*i.e.* miR-155 and miR-18a), cirrhosis (miR-217) and alcohol (miR-126, miR-199 and miR-200).[20,21] miR-122, miR-124, miR-125a-b, and miR-199 are the most studied and they have been frequently found to be involved in the carcinogenesis, angiogenesis and metastases of HCC. Several studies evaluated the possible role of miRNA signature in identifying HCC with metastatic potential and higher recurrence rate. A number of data suggested a poor prognosis in HCC patients whose tumors express some specific miRNAs (miR-122, miR-26, miR-22), and moreover, these tumors are also related to a worse response to therapy.[20] The potential role of miRNAs in HCC development, leads to a possible therapeutic application. In fact, cancer-related miRNAs can be inhibited by using miRNA antagonists, like antimiRs or antagomiRs. However, due to the complexity associated with pleiotropic miRNA functions and lncRNAs, the number of clinical trials exploring this technique is currently very limited[21] and more research still needs to be done to explore the potential benefits in clinical practice.

Microenvironment effect

Recently, a number of studies showed that a strong contribution to HCC heterogeneity and cancer cell proliferation is played by tumor microenvironment. Recent evidences are in favor of a tight interaction between tumor cells and stroma cells, such as macrophages, activated stellate cells, endothelial cells, immune cells and platelets.[22] In particular, since host immune response suppression results in the unopposed development of HCC, activation of the host immune system may serve as a potential treatment strategy yielding improved outcomes. Several immunotherapies are still in the pre-clinical or clinical trial phase and require further studies to develop effective therapeutic strategies for HCC. For the purpose of this review, we will not go in the details of immunotherapy for HCC.

Conclusions

Resuming, present treatment modalities like surgical resection, chemotherapy, radiotherapy and local ablation techniques are yet inadequate and indicate the need for more effective treatments. Despite it has been very challenging to identify specific tumor drivers in HCC, translational research can contribute to identify possible pathways and immunotherapeutic targets involved in this process (Table 1).[23-31] The complexity of these data reinforces the critical role of translational research in this setting to clarify the exact process of HCC evolution.

Sorafenib is yet considered as the treatment of choice for patients with advanced disease, and regorafenib is the only systemic treatment able to provide survival benefit in HCC patients progressing on sorafenib treatment. Other drugs tested in different trials failed to demonstrate any benefit, highlighting that HCC has low sensitivity to chemotherapy that is in great part caused by multidrug resistance.

Immunotherapy for HCC is a new challenging treatment option and, in clinical trials, it consists of immune checkpoint inhibitors/antibody-based therapy and peptide-based vaccines.

Another challenging and very promising approach is microRNA-based therapy. It can be performed using two strategies. The first aims to inhibit oncogenic miRNAs by using miRNA antagonists and the second strategy is miRNA replacement, which involves the reintroduction of a tumor-suppressor miRNA mimetic to restore a loss of function.

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