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Review

Gene signatures in hepatocellular carcinoma (HCC)

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ABSTRACT

Primary hepatocellular carcinoma (HCC) is a significant human cancer globally, with poor prognosis. New and efficacious therapy strategies are needed as well as new biomarkers for early detection of at-risk patients. In this review, we discuss select microarray studies of human HCCs, and propose a gene signature that has promise for clinical/translational application. This gene signature combines the proliferation cluster of genes and the hepatic cancer initiating/stem cell gene cluster for identification of HCCs with poor prognosis. Evidence from cell-based assays identifies the existence of a mechanistic link between these two gene clusters, involving the proliferation cluster gene polo-like kinase 1 (PLK1). We propose that PLK1 is a promising therapy target for HCC.

1. Introduction

Primary liver cancer, hepatocellular carcinoma (HCC), is the fifth most common cancer world-wide [1]. In the Unites States, liver cancer, relative to other cancers, has the most rapidly growing mortality rate [2]. Major etiologic agents in HCC pathogenesis are chronic infection with hepatitis B virus or hepatitis C virus [3,4,5]. Other causal factors of lower incidence include alcohol abuse, metabolic disorders, and environmental agents, e.g., exposure to aflatoxin B1 [6]. Regarding HBV-mediated HCC, despite availability of the HBV vaccine, the World Health Organization estimates that globally 400 million people are chronically infected with HBV. Moreover, the HBV vaccine is not always protective and children born of infected mothers also become chronically infected. There is no vaccine for HCV. Current treatments for chronic HBV infection include antiviral nucleoside analogs that eventually result in viral resistance [7]. Treatment for HCV infection includes a combination of interferon and ribavirin [8]. When diagnosed at early stage, HCC remains eligible for potential curative options such as surgical resection, orthotopic liver transplantation or percutaneous destructions. However, most of HCCs have widespread dissemination within the liver at diagnosis (intermediary stage) or show extrahepatic dissemination within the portal tract, lymph nodes or distant visceral metastasis (advanced stage) [9]. Either transarterial hepatic chemoembolization for intermediary stage HCCs, or systemic targeted therapies such as sorafenib – i.e. the anti-angiogenic and anti-MAPK pathway agent – for advanced stage HCCs, are of modest, although significant benefit [10]. As recommended by Llovet and Bruix [11], new and efficacious therapies are needed, along with new diagnostic biomarkers for early detection of liver cancer.

Microarray studies of human tumor samples and bioinformatics meta-analyses continue to provide a wealth of information regarding genes differentially expressed in various types of cancer [12–16]. For liver cancer, more than 300 microarray studies have been published [17] identifying genes deregulated in HCC, although some of the published studies provide more transparent data than others. The ongoing challenge is to identify and characterize clinically relevant genes that can serve as early biomarkers for detection and classification of the disease or serve as targets for designing mechanism-based therapies. This review focuses on select microarray studies of human liver tumors. Herein we highlight specific HCC gene signatures we consider promising for translational application in diagnosis of at-risk patients. We base this assessment on the link of the proposed HCC gene signatures to established mechanisms of cancer pathogenesis as well as to liver physiology and development. We will present "cancer" and "liver-specific" gene signatures associated with HCC pathogenesis.

1.1. Cancer gene signature: the proliferation gene cluster

Chen et al. in 2002 [18] analyzed 102 primary HCC tumor samples and 74 non-tumor samples for differentially expressed genes, using a cDNA microarray representing 17,400 human genes.

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They demonstrated increased expression of the proliferation cluster of genes required for cell cycle progression. Up-regulated genes include those involved in DNA replication, e.g., the minichromosome maintenance3-7 (MCM3-7) proteins, thymydilate synthase (TYMS), proliferating cell nuclear antigen (PCNA), and those involved in G2/M progression, such as mitotic kinases CDC2, CDC20, polo-like kinase 1 (PLK1), and mitotic regulators MAD2 and Bub1. Also, they observed decreased expression of liver-specific genes, indicative of hepatocyte de-differentiation and/or loss of liver function. This proliferation gene signature distinguished liver tumors with mutant p53 from those with wild type (WT) p53, and liver tumors with vascular invasion.

In 2004, Thorgeirsson's group [19] analyzed the gene expression profile of 91 human primary HCC tumors by microarray analyses. They also identified enhanced expression of the proliferation cluster of genes as the best predictor for an unfavorable outcome. This proliferation gene signature convincingly distinguished two groups of HCC patients, Clusters A and B, having significant differences in survival of 30 months vs. 90 months, respectively. The proliferation cluster of genes included: PCNA, Bub3, MCM2, 6, and 7, and cell cycle regulators cyclinA2 (CCNA2), cyclinB1 (CCNB1), CDC2 associated protein2 (CKS2), and cyclin-dependent kinase 4 (CDK4). Additional features of the poor survival HCC Cluster A include reduced expression of liver specific genes, similar to the observations by Chen et al. [18], and enhanced expression of genes involved in proteasomal degradation and the ubiquitin pathway. The ubiquitin pathway is often deregulated in cancer [20]. Together, these genes were termed the survival gene expression signature.

In a subsequent study [21], Thorgeirsson's group identified by global gene expression analyses of 139 human HCCs two subtypes of HCC. The one subtype exhibited features of hepatoblasts (HB) and the other of differentiated hepatocytes (HC). The strategy for this study involved comparison of gene expression profiles from three different species (human, rat and mouse). Specifically, the gene expression profiles of human HCCs were compared to those of fetal and adult rat hepatocytes, and to mouse HCCs that originated from adult hepatocytes. The mouse hepatocyte-originating HCCs were obtained from the Myc/E2F1 and Myc/TGFα mouse liver cancer models. In these animal models transgenes were expressed from the albumin promoter which is transcriptionally active in differentiated hepatocytes. Importantly, the gene expression profile of rat hepatoblasts in comparison to that of mouse hepatocytes (from the two mouse HCC models) was distinct and well-separated from each other. Interestingly, the gene expression profiles of several human HCC samples co-clustered with rat hepatoblasts suggesting a similar cell developmental stage. This subtype was referred to as the HB subtype and was shown to express markers of hepatic progenitors or "oval cells," including keratin 7 (KRT7) and keratin 19 (KRT19). Both HB and HC subtypes expressed α-fetoprotein (AFP) to similar levels. Further analysis of the gene expression profiles by hierarchical cluster analyses, demonstrated that the HB subtype co-clustered with the proliferation group of genes which characterized the poor survival Cluster A of human HCCs [19]. The HC subtype was further subdivided into Cluster A (proliferation signature-positive) and Cluster B (proliferation signature-negative). The poorest survival between HB and HC subtypes in Cluster A was exhibited by the HB subtype.

Interestingly, another microarray study by the Zucman-Rossi group [22] came to the same conclusion. Specifically, this microarray analysis also identified two major clusters of HCC tumors which were sub-grouped into six distinct subgroups (G1–G6). Significantly, these G1–G6 subgroups were also characterized by their association with distinct clinical and molecular/genetic alterations, including viral infection, activation of the PI3K/AKT pathway and p53 mutations, thereby providing additional descriptors for precise classification of HCCs. Subgroups G1-G3 exhibited high rate of chromosomal instability and were associated with poor prognosis. Importantly, HCC subgroups G1-G3 over-expressed proliferation, cell cycle and DNA metabolism genes, similar to the cluster A subtype of HCCs described by Lee et al. [19]. Interestingly, subgroups G1 and G2 that originated from patients with chronic HBV infection, low HBV copy number vs. high, respectively, also exhibited over-expression of fetal genes including AFP and parentally imprinted genes, thus resembling the poor prognosis, HB cluster A subtype [21].

Table 1
Proliferation cluster gene signatures identified using human HCCs [18,19] and c-myc up-regulated proliferation genes [24]. Green designates the overlap of the proliferation cluster gene signatures in the studies by Chen et al. [18] and Yu et al. [24]. Orange designates overlap of the proliferation cluster gene signatures in Lee et al. [19] and Yu et al. [24]. Blue designates overlap of proliferation genes from the three studies [18,19,24]. Red designates overlap between the study by Segal et al. [27] and genes found in the other three columns.

Chen et al. [18]			Yu et al. [24]	Lee et al. [19]
AURKA	CDK1	MCM6	AURKA	BUB1/BUB3
BIRC5	CLK2	PCNA	BIRC5	CCNB2
BRCA2	E2F1	PLK1	BRCA2	CKS2
BUB1/BUB3	E2F3	RRM1	BUB1/BUB3	HMGN2
CCNA2	E2F5	TOP2A	CCNB2	KIF20A
CCDN1	E2F8	TYMS	CKS2	PCNA
CDC2	FOXM1	USP1	CDKN3	TOP2A
CDC7	GMNN	UBE2S	E2F8	USP1
CDC14	IGF1		HMGN2	UBE2S
CDC20	IGFBP3		IDI1	
CDC25B	JUNB		KIF20A	
CDCKN2C	IDI1		PCNA	
CDKN3	MAPK7		PLK1	
CDK4	MAPK13		RRM1	
CDK5	MCM3		TOP2A	
CDK7	MCM4		USP1	
CDK5R1	MCM5		UBE2S	

Representative Proliferation Cluster Genes Segal et al. [27]						
AURKB	CCNB2	CKS2	IGF2	MAP4K4	MCM6	PCNA
BUB1B	CDC2	E2F3	IGF2BP3	MAPK6	MCM7	PLK1
CCNA2	CDC20	E2F5	JUNB	MCM2	MYC	TOP2A
CCNB1	CDC42EP1	FOXM1	MAP2K3	MCM3	PARP2	TYMS

1.2. Proliferation gene signature and polo-like kinase 1 (PLK1)

The proliferation gene signatures identified by Chen et al. [18] and Lee et al. [19,21] overlap the c-myc up-regulated gene signature identified using the non-transgenic mouse model of B-cell lymphoma [23]. The significance of this animal model is that the oncogenic transformation of p53-null bone marrow cells occurred in vivo, following infection with a conditionally active c-myc encoding retrovirus [23,24]. Accordingly, this experimental design permitted identification of a broader set of proliferation genes, those expressed at earlier times in the process of oncogenic transformation. The relevance of this animal model to liver cancer is that c-myc over-expression also characterizes human HCC [18,19,25], including hepatoblastoma, a rare liver cancer in children [26]. Furthermore, the proliferation cluster is shared across diverse human malignancies indicating a common mechanism in tumor progression [27]. Table 1 shows the proliferation cluster of genes identified in human HCCs by the studies of Chen et al. [18] and Lee et al. [19]. The central column (Table 1) includes c-myc up-regulated proliferation genes identified by Yu et al. [24] in the non-transgenic mouse model of B-cell lymphoma. In addition, Table 1 shows the proliferation cluster of genes identified by Segal et al. [27] via meta-analysis of 1975 microarrays from multiple types of human tumors.

In HBV-mediated hepatocarcinogenesis, studies in animal models [28,29] have demonstrated that the viral X protein acts as a