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Review

Emerging histopathological prognostic biomarkers in hepatocellular carcinomas

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ABSTRACT

Purpose: Hepatocellular carcinoma (HCC) is one of the most common malignant neoplasms and is associated with a poor survival rate. An increased understanding of the molecular mechanisms underlying HCC carcinogenesis and progression has enabled the identification of many potential HCC biomarkers. In addition to predicting prognosis and recurrence, these markers may guide decisions regarding therapeutic intervention of potential targets and appropriate therapeutic modalities for individual patients. Considering the high recurrence rate associated with HCC resection, improved biomarkers that can be used for early diagnosis, predicting recurrence, and monitoring progression are urgently needed in clinical practice.

Study section and results: This systematic review examines evidence from published studies that have reported emerging prognostic biomarkers, paying particular attention to markers evaluated by histopathological analysis of resected HCC tissues. These markers include molecules involved in cellular proliferation and survival (*e.g.* cyclins and cyclin dependent kinase inhibitors, mutant p53, and hepatocyte growth factor receptor [c-MET]), aberrantly expressed cell surface proteins (*e.g.* glypican 3, monocarboxylate transporter 4, and hepatocyte growth factor activator inhibitor type 1), and factors found in altered tumor microenvironments (*e.g.* angiogenesis factors, regulatory T-cells, tumor-associated macrophages, and hepatic stellate cells).

Conclusion: Identification of novel, effective biomarkers for the diagnosis and prognosis of HCC is critical for the improvement of companion diagnostics in personalized oncology therapies for HCC. The molecules described in this review are attractive candidates for future HCC biomarkers to be used in clinical oncology practice.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer-related death worldwide. The incidence of HCC is almost equal to its death rate, and underlying chronic liver disease is a major obstacle to the improvement of treatment outcomes in HCC patients. Cirrhosis is a major risk factor for the development of HCC, ~80% of which is associated with chronic hepatitis B virus (HBV) and/or hepatitis C virus (HCV) infection [1]. Other known risk factors for HCC development include chronic alcohol consumption, aflatoxin B1 exposure, diabetes, non-alcoholic fatty liver disease, and some inherited metabolic disorders such as hereditary hemochromatosis and porphyria cutanea tarda [2,3]. Despite progress in surgical and non-surgical therapies, the prognosis for HCC continues to trail behind those of other common human cancers, and HCC remains resistant to conventional chemotherapy and radiotherapy. Although the multikinase inhibitor sorafenib has extended the survival of HCC patients by approximately three months [4], there is a critical need for the discovery of novel potential targets for therapeutic intervention and the development of additional treatment modalities. In this regard, new potential target molecules expressed in HCC tissues and significantly related to patient prognosis are urgently needed.

We have previously reported novel prognostic markers expressed on the HCC cell surface, such as hepatocyte growth factor activator inhibitor type 1 (HAI-1) [5], glypican-3 (GPC3) [6], and

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monocarboxylate transporter 4 (MCT4) [7], as determined by immunohistochemical studies of surgically resected HCC tissues. We showed that HAI-1 and MCT4 are independent poor prognosis factors in HCC patients. GPC3 has also been reported by other groups to be a prognostic marker in HCC patients [8,9], and our study suggested that its circumferential membranous expression in particular is related to poor prognosis [6]. These factors may have clinical implications for the development of novel targeted molecular HCC therapies and improved prediction of patient outcomes in clinical practice. The aim of this review is to examine the current knowledge regarding prognostic markers of HCC. A systematic literature review was performed using PubMed databases of English language publications and the following keywords in various combinations: "hepatocellular carcinoma," "prognosis," "molecular markers," and "biomarkers". The prognostic values of the listed markers are discussed, and our recent data on three emerging HCC cell surface biomarkers, GPC3, HAI-1, and MCT4, are also described.

2. Conventional prognostic factors in HCC

Various clinicopathological prognostic factors in HCC have been established and used in clinical practice. Well-studied poor prognosis factors in HCC include advanced age [10,11], male gender [12–14], high Child-Pugh score [15], low indocyanine green disappearance [16], low serum albumin [17], infiltrative gross appearance of tumor [18,19], large tumor size [10,16,20], number of tumors [10,15,16], high pathological grade and node-metastasis stage [21], large nuclear area (>50 μ^2) [22], portal vein invasion [10,15,20], cirrhosis [10], and positive hepatectomy margin status [23]. It is obvious from these factors that poor tumor prognosis is related not only to malignant cancer cell phenotypes such as marked cellular atypia, invasion, and metastasis, but also to decreased liver function.

Alpha-fetoprotein (AFP) and des-gamma-carboxyprothrombin (DCP) have been widely used as serum markers. AFP is an oncofetal glycoprotein produced primarily by the fetal liver and repressed in adults. Serum AFP remains the most useful tumor marker for HCC screening, with high AFP concentrations (>400 ng/mL) predicting a poor prognosis [10,15]. However, since the diagnostic sensitivity of AFP for early HCC is only 39–64% [24], additional serum biomarkers for detecting early HCC are desired. AFP can be divided into three different forms, namely AFP-L1, AFP-L2, and AFP-L3, according to their lectin lens culinaris agglutinin binding. Among these, the AFP-L3 fraction is highly specific for HCC, and is related to poor tumor differentiation and portal vein invasion [25], thus making AFP-L3 an independent prognostic factor of disease free survival (DFS) [26]. DCP, also known as protein induced by vitamin K absence or antagonist II (PIVKA-II), is an abnormal form of prothrombin that shows incomplete carboxylation of its glutamic acid residues. Like serum AFP, serum DCP has been used as a diagnostic HCC biomarker, with increased DCP levels relating to a high recurrence rate after HCC resection [27]. NX-PVKA, a DCP variant containing fewer glutamic acid residues, is detected by the P-11 and P-16 monoclonal antibodies and has been reported as an independent poor prognosis factor [28]. The DCP/NX-PVKA ratio is a useful marker for the identification of HCC in patients taking vitamin K antagonists such as warfarin [29].

3. Cellular prognostic markers of HCC

3.1. Markers of proliferating activity

Mitotic index is related to poor prognosis in HCC patients [30]. Therefore, various molecular markers for the quantification of cancer cell proliferation activity have been used for prognostic evaluation. Currently the major markers are Ki-67 and proliferating cell nuclear antigen (PCNA) [31].

Ki-67 nuclear antigen is widely used as a proliferation-specific marker. Its presence during active phases of the cell cycle, the G1, S, and G2–M phases, and absence in the resting G0 phase make it an excellent marker for determining the growing fraction of a given cell population [32]. PCNA was originally identified as a 36-kDa nuclear antigen whose expression is associated with proliferating cellular phenotypes [33]. In HCC, the fraction of Ki-67- or PCNA-positive tumor cells (Ki-67/PCNA labeling index; Ki-67/PCNA-LI) is correlated with aggressive tumor growth rate, and patients with higher Ki-67-LI or PCNA-LI levels show poor overall survival (OS) and DFS rates [34,35].

3.2. Cell cycle regulators and tumor promoter or suppressor genes

Cyclins and cyclin-dependent kinase inhibitors (CKIs) have been reported as prognostic factors in HCC patients. Cyclin proteins control the progression of the cell cycle by activating cyclin-dependent kinases. On the other hand, CKIs such as p15 (*CDKN2B*), p16 (*CDKN2A*), p18 (*CDKN2C*), p21 (*CDKN1A*), and p27 (*CDKN1B*) are potent negative cell cycle regulators. Cyclins relating to poor prognosis in HCC patients include cyclins A, D1, and E [36,37]. In multivariate analyses, gain of p16 and loss of p18 expression predicted increased and decreased OS, respectively [38,39]. HCCs expressing low p27 levels are also related to poor prognosis [38].

The *p53* tumor suppressor gene is responsible for regulating the cell cycle at the G1/S and G2/M interfaces, and also induces apoptosis in response to severe genetic damage. Dysfunction of p53 may induce abnormal cell growth, increased cell survival, genetic instability, and drug resistance. Mutations of *p53* frequently result in an intracellular accumulation of mutant p53 protein, which is immunohistochemically detectable as p53 overexpression. Sheen et al. showed that mutant *p53* gene products were present in >80% of hepatectomy specimens [40]. Although conflicting results have been reported in prognostic studies of HCC patients [41], p53 overexpression is one of the most significant factors associated with poor overall survival rates of HCC patients after resection [42]. The presence of *p53* mutations was also associated with a shortened OS and DFS [43]. Consequently, measurement of serum p53 using anti-p53 antibody could also provide a useful prognostic factor for HCC patients [44]. Mouse double minute 2 homolog (MDM2) is a ubiquitin protein ligase that inactivates p53, while *MDM2* gene transcription is regulated by p53. In HCC cells, MDM2 intranuclear immunoreactivity showed a significant positive correlation with p53 expression [45], however a prognostic study of MDM2 in patents with HCC is not available.

3.3. Micro RNAs (miRNAs)

Micro RNAs are small non-coding RNA molecules that control target gene expression and are implicated in the regulation of diverse cellular pathways. In terms of negative regulators of HCC, growth inhibitory roles have been reported for miR-198 [46], miR-219-5p [47], miR-138 [48], and miR-214 [49], which target the hepatocyte growth factor (HGF)/c-MET pathway, GPC3, cyclin D3, and beta-catenin, respectively. An anti-invasive role was reported for miR-338, likely through targeting of the hedgehog signaling pathway protein, Smoothened (SMO) [50]. On the other hand, miRNAs promoting a malignant HCC phenotype include miR-224 [51], miR-17-5p [52], miR-18b [53], and miR-106b [54]. Among