

Autoantibodies against tumor-associated antigens for detection of hepatocellular carcinoma

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Author contributions: Hong Y wrote the manuscript; Huang J modified the manuscript.

Supported by A grant from the National Natural Science Foundation of China, No. 81071973; A grant from the Scientific Research Foundation for Returned Overseas Chinese Scholars, Bureau of Human Resources and Social Security of Beijing, China (Key project, 2010).

Conflict-of-interest: We declare that we have no conflict of interests, and we have no financial and personal relationships with other people or organizations that can inappropriately influence our work.

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Received: December 10, 2014
Peer-review started: December 13, 2014
First decision: January 8, 2015
Revised: January 30, 2015
Accepted: April 16, 2015
Article in press: April 20, 2015
Published online: June 18, 2015

Abstract

Hepatocellular carcinoma (HCC) is one of the most common tumors worldwide. The survival rate after the onset of symptoms is generally less than one year for the late presentation of HCC, and reliable tools for early diagnosis are lacking. Therefore, novel biomarkers for the early detection of HCC are urgently required. Recent studies show that the abnormal release of proteins by tumor cells can elicit humoral immune responses to self-antigens called tumor-associated antigens (TAAs). The corresponding autoantibodies can be detected before the clinical diagnosis of cancer. Therefore, there is growing interest in using serum autoantibodies as cancer biomarkers. In this review, we focus on the advances in research on autoantibodies against TAAs as serum biomarker for detection of HCC, the mechanism of the production of TAAs, and the association of autoantibodies with patients' clinical characteristics.

Key words: Hepatocellular carcinoma; Diagnosis; Serological marker; Autoantibody; Tumor associated antigen

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Core tip: There is growing interest in using serum autoantibodies as cancer biomarkers. However, the mechanism and clinical association of autoantibodies in hepatocellular carcinoma (HCC) remains unclear. In this review, we focus on the advances in research on autoantibodies against tumor-associated antigens (TAAs) as serum biomarker for detection of HCC, the mechanism of the production of TAAs, and the association of autoantibodies with patients' clinical characteristics.

Hong Y, Huang J. Autoantibodies against tumor-associated

antigens for detection of hepatocellular carcinoma. *World J Hepatol* 2015; 7(11): 1581-1585 Available from: URL: <http://www.wjgnet.com/1948-5182/full/v7/i11/1581.htm> DOI: <http://dx.doi.org/10.4254/wjh.v7.i11.1581>

INTRODUCTION

Liver cancer is the sixth most common malignant disease worldwide, and approximately 50.5% of new cases and 51.4% of cancer-related deaths occur in China^[1]. The survival rate after the onset of symptoms is generally less than one year for the late presentation of hepatocellular carcinoma (HCC), and reliable tools for early diagnosis are lacking.

Ultrasound is recommended as a screening tool for early detection of HCC, although it is not very sensitive and is highly operator dependent. Computed tomography is not recommended as a screening tool for HCC because of radiation exposure^[2,3]. One current focus of HCC research is the development of a blood test to aid in the diagnosis of this disease. Many serologic biomarkers of HCC are available, including alpha-fetoprotein (AFP), des- γ -carboxyprothrombin, lens culinaris agglutinin-reactive AFP-L3^[4,5], Dickkopf-1^[6], and squamous cell carcinoma antigen^[7]. To date, AFP is the only serum biomarker available for HCC surveillance. However, AFP does not yield satisfactory results to diagnosis HCC in its early stages. Specifically, using a cutoff value of 20 ng/mL, the sensitivity and specificity of AFP assays range between 41%-65% and 80%-90%, respectively, and the sensitivity is lower when AFP is used to detect early-stage HCC^[8]. Therefore, novel serum biomarkers that detect HCC before symptoms are apparent are urgently required.

The immune system is the first line of defense against pathogens. During the earliest stage of tumorigenesis, proteins released by tumor cells, or peptides at the surface of tumor cells, can elicit humoral immune responses against the tumor and are therefore called tumor-associated antigens (TAAs). The production of TAAs is not completely understood. The proteins are likely mutated, overexpressed, posttranslationally modified, misfolded, aberrantly cleaved, or aberrantly localized in tumor cells^[9]. Therefore, autoantibodies against TAAs are readily isolated, because they are secreted, and their titers increase in response to robust biological amplification of TAAs. Most important, TAAs can be detected before the clinical diagnosis of cancer. Further, unlike polypeptides, antibodies are highly stable in serum and are not proteolyzed. The half-life of TAAs in the bloodstream ranges between 7-30 d, depending on the subclass of immunoglobulin, and may persist for as long as the immunizing autoantigen, which simplifies sample preparation^[10]. Thus, detection of anti-TAA autoantibodies will be easier than detecting TAAs themselves, suggesting that the measurement of anti-TAA antibodies may offer the potential to improve

upon assays employing conventional biomarkers^[11]. For example, Li *et al.*^[12] reported that the elevated levels of serum antibodies against insulin-like growth factor-binding protein-2 allowed detection of early-stage cancers.

In this review, we discuss advances in research on autoantibodies against TAAs as biomarkers for the detection of HCC, with particular focus on the mechanism of the production of TAAs and the association of autoantibodies with clinical parameters.

ANTI-TAA AUTOANTIBODIES IDENTIFIED IN PATIENTS WITH HCC

The number of reports of TAAs in patients with HCC has recently increased. The main TAAs reported since 1993 are listed in Table 1. Among them, the tumor suppressor protein p53 is one of the most highly immunogenic TAAs identified to date. The prevalence of serum anti-p53 antibodies among HCC patients ranges from 12.2%-73.07%^[13-15]. The reasons for the differences are unknown but may be caused by unidentified biological and geographical differences in study populations. Except for HCC, antibodies against p53 are present in patients with many types of cancer and may provide a tool for detection of cancer recurrence^[16].

The insulin-like growth factor mRNA-binding (IMP) family member IMP2 binds to mRNA and regulates translation of the mRNA that encodes insulin-like growth factor 2 and is frequently reported as a TAA in patients with HCC^[17-19]. Members of the IMP family are oncofetal proteins, which disappear from all tissues soon after birth but are frequently re-expressed during the malignant transformation of numerous cell types. IMP2 was first identified as a TAA for HCC in 1999^[20]. An autoantibody against IMP2 is present in 21% of patients with HCC patients, but is undetectable in precursors such as chronic hepatitis and liver cirrhosis^[20].

Elevated levels of autoantibodies to calreticulin^[21], cyclin B1^[22], centromere protein F (CENPF)^[23], and survivin^[24,25] are frequently detected in the sera of patients with HCC. However, no autoantibody binds its immunogen with enough sensitivity to detect HCC^[26]. To overcome this drawback, multi-autoantibody panels were applied to improve sensitivity. For example, Zhang and colleagues^[27] constructed an antigen microarray comprising IMP1, IMP2, IMP3, p53, c-myc, cyclin B1, survivin, and p16, and the results show that the frequency of antibody detection to any individual TAA of patients with HCC varied from 9.9%-21.8%. With the successive addition of TAAs of all eight antigens, there was a stepwise increase in positive antibody reactions, reaching a frequency of 59.8% in an entire cohort. This shows that a mini-array of eight TAAs enhanced antibody detection for the diagnosis of HCC. When Sui1 and RalA were added to the panel, the final cumulative prevalence of anti-TAA antibodies increased to 66.2% (51/77)^[28]. Therefore, multi-autoantibody panels might

Table 1 Tumor-associated antigens detected in patients with hepatocellular carcinoma reported since 1993

| Ref. | TAAs reported |
|---|---|
| Yau <i>et al</i> ^[36] | hnRNP L |
| Akada <i>et al</i> ^[42] | HSP70, SOD2, and PRDX6 |
| Shao <i>et al</i> ^[41] | Glucose-regulated protein 78 |
| Nomura <i>et al</i> ^[39] | Ku86 |
| Liu <i>et al</i> ^[40] | CENPF, DDX3, HSPA4, HSPA5, VIM, LMNB1, and p53 |
| Pekáriková <i>et al</i> ^[21] | CRT |
| Chen <i>et al</i> ^[28] | Sui1, RalA |
| Wang <i>et al</i> ^[43] | KRT23, AHSG and FTL |
| Wang <i>et al</i> ^[44] | RalA |
| Looi <i>et al</i> ^[45] | HSP60, HSP70 |
| Li <i>et al</i> ^[35] | DDX3, eEF2, AIF, hnRNP A2, PBP, and TIM |
| Chen <i>et al</i> ^[46] | EIF3SI, LDHA, RFC2, and MCART1 |
| Zhang <i>et al</i> ^[27] | IMP1, IMP2, IMP3, p53, c-myc, cyclin B1, survivin and p16 |
| Akere <i>et al</i> ^[13] | p53 |
| Zhou <i>et al</i> ^[47] | HCC-22-5 |
| Takashima <i>et al</i> ^[48] | HSP70, GAPDH, PRX, Mn-SOD |
| Looi <i>et al</i> ^[49] | p16 |
| Yagihashi <i>et al</i> ^[25] | Survivin |
| Su <i>et al</i> ^[17] | IMP2 |
| Himoto <i>et al</i> ^[19] | IMPs |
| Himoto <i>et al</i> ^[18] | IMPs, p53, c-myc, and survivin |
| Zhang <i>et al</i> ^[24] | c-myc, cyclin B1, IMP1, Koc, p53, IMP2, and survivin |
| Soo Hoo <i>et al</i> ^[50] | p53, IMP2, Koc, CENP-F, p90 |
| Le Naour <i>et al</i> ^[51] | CRT, CK8, NDK-A, and ATP5B |
| Zhang <i>et al</i> ^[23] | IMP2, CENPF |
| Zhang <i>et al</i> ^[20] | IMP2 |
| Raedle <i>et al</i> ^[52] | p53 |
| Covini <i>et al</i> ^[22] | Cyclin B1 |
| Imai <i>et al</i> ^[53] | HCC1 |

TAAs: Tumor-associated antigens; HCC: Hepatocellular carcinoma; IMP: Insulin-like growth factor mRNA-binding; CENPF: Centromere protein F.

be useful tools for HCC diagnosis.

A feature of HCC is that antecedent liver cirrhosis and chronic hepatitis are common precursors, and 80%-90% of patients with cirrhosis develop HCC^[29]. Autoantibodies to TAAs are detected during the transition to malignancy^[30]. It was proposed that these antibody responses might be stimulated by cellular proteins that are involved in carcinogenesis. Thus, cirrhosis-associated autoantibodies can identify individuals at risk of developing HCC.

MECHANISM OF THE PRODUCTION OF AUTOANTIBODIES AGAINST TAAs

The mechanism of generation of autoantibodies against TAAs is not fully understood. TAA proteins are likely either mutated, overexpressed, or aberrantly localized in tumor cells^[9]. Autoantibodies may be elicited by proteins with incorrect posttranslational modifications that are recognized as nonautologous^[31]. Phosphorylation, glycosylation, oxidation, or proteolytic cleavage may generate a neo-epitope with affinity for the major histocompatibility complex or T-cell receptor that induces an immune response^[32]. For example, HSP60 localizes mainly to mitochondria, but in tumor cells it is present

in the cytoplasm and cell membrane, leading to the induction of an autoimmune response^[33]. Moreover, the level of expression of HSP60 is significantly higher in breast tumor tissues, suggesting that overexpression of HSP60 may represent a mechanism of developing immunogenicity in patients with breast cancer^[33]. Similarly, our recent study shows that the high titer of anti-CENPF autoantibody in HCC serum is likely caused by an autoimmune reaction in response to overexpression of CENPF^[34].

ASSOCIATION OF THE PREVALENCE OF AUTOANTIBODIES WITH THE CLINICAL CHARACTERISTICS OF PATIENTS WITH HCC

There are relatively few studies on the clinical significance of autoantibodies in patients with HCC because of insufficient numbers of patients and the lack of accurate clinical information. There is evidence, however, showing that there are no statistically significant differences in patients with HCC in the prevalence of autoantibodies against DEAD box 3, eEF2, AIF, hnRNP A2, PBP, and TIM and patients' characteristics of sex, histological grade, or TNM classification^[35]. However, tumors > 5 cm in diameter are present more frequently in patients with anti-eEF2 autoantibodies compared with those with small tumors (> 5 cm in diameter) ($P < 0.05$)^[35]. The rates of detection of autoantibodies against AIF and hnRNP A2 in patients with HCC without regional lymph node metastasis were significantly higher compared with those with regional lymph node metastasis ($P < 0.05$)^[35]. There is a significant difference in size of tumors of patients with HCC cases that correlates with prevalence of autoantibodies against hnRNP L-67-88, with the average tumor size of 5.84 ± 4.23 cm in patients with detectable autoantibodies whereas 3.70 ± 2.07 cm in patients without detectable autoantibodies^[36]. Survival analysis shows that the survival rates of patients with hepatitis B virus-positive HCC with autoantibodies are significantly lower compared with those without detectable autoantibodies ($P < 0.05$), indicating that an elevated level of autoantibody against hnRNP L-67-88 is associated with larger tumors and poorer prognosis^[36].

In our recent study (data not shown), analysis of clinicopathological associations shows that the prevalence of positive for autoantibodies against CENPF and HSP60 is higher in patients with HCC < 50 years of age. The prevalence of autoantibodies against CENPF is higher in patients with well-differentiated HCC with Child-Pugh grade A liver function. In contrast, there are no data available, to our knowledge that associates autoantibodies against p53 with patients' clinical characteristics. In patients with colorectal cancer (CRC), there is an increase in the prevalence of anti-p53 autoantibodies in carcinoma *in-situ* (6%) compared with adenomas (1%), indicating that the level of anti-p53 autoantibody increases with CRC

progression^[37]. However, almost all studies report that there is no association between anti-p53 autoantibodies and CRC stage progression^[37], and only a handful of studies suggest an association between anti-p53 autoantibody and T-stage, selected nodal disease, and metastases^[37], suggesting that the autoantibody may have more value in the early diagnosis of cancer than for prognosis. However, subanalysis of autoantibody detection rates in tumors of different causes or stage was not possible in many studies, because of unknown cause or lack of tumor-stage data of many of the HCC samples^[38].

PROSPECTS

During the past few years, the potential utility of autoantibodies against TAAs as biomarkers for HCC has been explored. However, their value for this purpose is controversial. There is concern that there is no single anti-TAA autoantibody with high sensitivity and specificity that detects HCC, and no large-scale clinical trial has been conducted to validate candidate TAAs^[26,28,35,39-41]. Further studies of large populations with precise clinical information should be conducted to determine whether autoantibodies to TAAs are associated with patients' clinical characteristics as well studies on the mechanism of the production of TAAs, with the aim of clarifying the role of specific TAAs as biomarkers for the early diagnosis and prognosis of HCC.

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P- Reviewer: Hann HW, Wang GY S- Editor: Gong XM

L- Editor: A E- Editor: Liu SQ





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