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Advances in Early Diagnostic Markers for Hepatocellular Liver Cancer

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Abstract: Hepatocellular carcinoma (HCC) is a common malignancy in China, and its incidence and lethality are increasing every year, so it is especially important to choose cost-effective diagnostic methods to achieve early diagnosis and treatment. At present, the most widely used examination measures in the world are ultrasonography of the liver and tumor marker alpha-fetoprotein (AFP), but its accuracy is limited, the research on tumor markers of HCC has been progressing rapidly in recent years, and this paper summarizes the roles and values of protein markers, enzyme and its isozymes markers, and gene markers in the identification of early hepatocellular carcinoma.

Keywords: Hepatocellular Carcinoma; Early Diagnosis; Tumor Markers.

1. Introduction

Hepatocellular carcinoma is a common malignant tumor of the digestive system, with about 906,000 new cases and 830,000 deaths worldwide each year. Its incidence and mortality rates in China rank fourth and second, respectively, and show an obvious rising trend [1, 2] . The main pathological types of liver cancer can be divided into hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma and mixed hepatocellular carcinoma. Among these types, hepatocellular carcinoma has the highest incidence, accounting for 75% to 85% of all cases. The onset of primary liver cancer is usually insidious, with no specific symptoms in the early stages and rapid progression of the disease, resulting in the majority of patients entering the locally advanced stage or developing distant metastasis by the time of diagnosis. According to statistics, the 5-year survival rate of patients with progressive liver cancer is only about 15%, while the 5-year survival rate of patients with early liver cancer can reach 75%. To improve the prognosis of patients, early detection and diagnosis are therefore important. The diagnosis of HCC is usually based on serum tumor marker tests, imaging tests and pathologic histology. Tumor marker tests offer several advantages, including noninvasiveness, economy, convenience, objectivity, operability, and the ability to dynamically monitor disease progression. In this paper, we present a review of the current research progress related to the application of tumor markers for early diagnosis of hepatocellular carcinoma.

2. Proteins

2.1. Alpha-fetoprotein Heterodimer 3 (alpha-Fetoprotein-L3, AFP-L3)

AFP is a single-chain glycoprotein and is currently the most commonly used tumor marker. Based on the difference in the binding affinity of its sugar chain to lentil lectin, AFP can be classified into three heterodimers: AFP-L1, AFP-L2, and AFP-L3, which increase in affinity in descending order. AFP-L3 as a percentage of total AFP (AFP-L3%) is commonly used as a screening indicator for primary liver cancer, and can detect small liver cancers with a diameter of < 2 cm in advance of imaging. AFP-L3 has a high heterogeneity but relatively low sensitivity in the diagnosis of early-stage liver cancer, and it is usually used in conjunction with other markers [3] .

2.2. Golgi Glycoprotein73 (GP73)

GP73 is a Golgi transmembrane glycoprotein mainly expressed in bile duct epithelial cells, and is hardly expressed in hepatocytes, but the expression of GP73 increases when hepatocytes are diseased. A foreign study included 352 HCC patients and found that their serum GP73 levels were significantly higher than those of cirrhotic patients, and GP73 has a higher sensitivity than AFP in the early diagnosis of HCC [4] , and a study conducted by ZHANG et al. [5] found that the sensitivity of GP73 in the diagnosis of HCC alone was 79%, and the specificity was 85%, and it is hoped that GP73 will become a potential new tumor marker for HCC. HCC tumor marker.

2.3. Phosphatidylinositol Proteoglycan 3 (gypican 3, GPC3)

GPC3 is a membrane protein located on the surface of cell membrane, composed of sugar, protein and lipid, and is a member of the phosphatidylinositol proteoglycan family. GPC3 plays an important role in embryonic development and is hardly expressed in normal adult tissues. In recent years, it has been found that the expression of GPC3 is significantly up-regulated in hepatocellular carcinoma, and its level fluctuates with the deterioration of the disease, so it is hypothesized that there is a certain correlation between GPC3 and the occurrence and development of HCC. Some studies have found that [6] , in early hepatocellular carcinoma, the specificity of serum GPC3 is 97.0%, but the sensitivity is only 55.1%, suggesting that GPC3 has the characteristics of high specificity and low sensitivity. And when GPC3 is combined with AFP and GP73, the sensitivity is 84.1% and the specificity is 71.7% [7] . Therefore, GPC3 can be used as part of the combined diagnosis.

2.4. Heat Shock Proteins (HSP90α)

Heat shock proteins are a class of proteins that act as molecular chaperones within cells. According to the degree of homology as well as molecular weight, heat shock proteins can be categorized into HSP110, HSP90, HSP70, HSP60, HSP40 small molecule HSPs and ubiquitin. Among them, HSP90 is an important family of heat shock proteins, and HSP90α is an isoform of HSP90, which is widely found in cells from microorganisms to mammals. Fu et al. [8] found that the sensitivity of Hsp90α in diagnosing hepatocellular carcinoma was 92.7%, and the specificity was 91.3%, and the sensitivities of Hsp90α compared with AFP were 92.4% and 57.5%, respectively, for patients with early-stage HCC. Compared with AFP, the sensitivity of Hsp90α for early HCC patients was 92.4% and 57.5%, respectively. It is obvious that Hsp90α is a very effective biomarker of liver cancer, which is of great significance for the early detection of HCC and the improvement of patients' prognosis.

3. Enzymes and Their Isoenzymes

3.1. Alpha-L fucosidase, AFU

AFU is a lysosomal hydrolase widely distributed in mammalian cells, which exists in the lysosomes of many mammalian cells as well as in blood and body fluids, and is involved in the metabolism of glycoproteins and glycolipids, and the activity of serum AFU is significantly elevated in patients with HCC. It has been found that early diagnosis of hepatocellular carcinoma relying on the detection of AFU can be made at least 6 months earlier than ultrasound diagnosis. [9] Some scholars in China have reported that the sensitivity of AFU used for the diagnosis of hepatocellular carcinoma is 81. The sensitivity of AFU for the diagnosis of liver cancer is 81.7%, and the specificity is 70.7%. [10] The activity level of AFU is related to the progression of the tumor, and a high AFU tends to suggest that the tumor is highly invasive and has a poor prognosis. The efficacy of using AFU alone to detect early HCC is not superior, and the combination of AFU with other markers can improve the diagnostic accuracy.

3.2. Desgamma Carboxyprothrombin (DCP)

DCP, also known as PIVKA-II, was first proposed as a marker for HCC by Libeman in 1984. DCP is an abnormal plasminogen produced by vitamin K deficiency or carboxylation of plasminogen precursor in hepatocytes, which lacks normal coagulation function. DCP has high sensitivity and specificity in the diagnosis of early-stage hepatocellular carcinoma, but it has the same problem of low sensitivity as AFP. DCP has high sensitivity and specificity in the diagnosis of early stage liver cancer, but DCP and AFP have the same problem of low sensitivity, although some studies have concluded that the combined detection of AFP and DCP does not improve the accuracy of HCC diagnosis [11]. However, most studies have concluded that the combination of AFP and DCP can greatly improve the diagnostic efficacy of HCC compared with a single marker test. Currently, DCP has been routinely used for the early diagnosis of hepatocellular carcinoma in Japan, and has been gradually used for clinical diagnosis in Europe and the United States, but it has not been widely used in China.

4. Genetic Marker

4.1. Micro RNA (miRNA)

miRNAs are a class of non-coding small molecule RNAs consisting of about 22 nucleotides, which are widely found in eukaryotes and are involved in the regulation of gene expression. miRNAs play an important role in tumorigenesis, and some of them are up-regulated in hepatocellular carcinoma to play the role of proto-oncogenes, e.g., miR-17-5p, miR21, miR-210, miR-221, miR-222. miR-224; others play the role of suppressor genes, e.g., miR-122, miR-192, miR-199, miR-101, let-7 family, miR-101, miR-7 family, and miR-224, miR-224; others act as oncogenes, such as miR-122, miR-192, miR-199, miR-101, let-7 family, miR-99a [12] . miRNAs have been shown to have unique expression patterns in a variety of tumors, including HCC. miRNA dysregulation is involved in all clinical stages of HCC, and can be used to distinguish HCC is involved in all clinical stages of HCC and can be used to differentiate HCC from healthy individuals and patients with other liver diseases. miRNAs are of high value in the diagnosis of HCC and can detect early HCC cases that are AFP negative. A combination of plasma miRNAs can effectively enable early diagnosis of HCC [13] . Some studies have found that the model consisting of seven miRNAs, miR-29a, miR-29c, miR-133a, miR-143, miR-145, miR-192, and miR-505, possesses high sensitivity and specificity in the diagnosis of early-stage hepatocellular carcinoma (HCC). PENG et al. [14] have found that the sensitivity and specificity of miRNAs, such as miR-130b and miR-150, in the diagnosis of HCC can be improved by the combination of plasma miRNAs. miR-130b, miR-150 and other miRNAs had a sensitivity and specificity of more than 80% in the diagnosis of HCC. Based on the high sensitivity and good stability demonstrated by miRNAs in early HCC diagnosis, their use as potential diagnostic markers can effectively make up for the shortcomings of ultrasonography and the lack of sensitivity of alpha-fetoprotein (AFP) detection. has an important clinical application in the early diagnosis and treatment of hepatocellular carcinoma, and it has become a current research hotspot in the field of molecular diagnosis. However, the short fragment characteristics of miRNA molecules, the high degree of similarity between different miRNA sequences, and their low concentration in body fluids pose technical challenges to the accurate quantitative detection of miRNAs. In addition, the higher detection cost also restricts the in-depth research and wide application of miRNAs in clinical diagnosis to a certain extent.

4.2. Circular RNA (circRNA)

CircRNA is a new type of endogenous non-coding RNA, which forms a covalently closed continuous loop with its head and tail, giving it a stable structure that is not easily degraded by nucleic acid ectonucleases, and its high tissue specificity gives it the potential to be used as a biomarker. aberrant expression of circRNAs plays an important role in the regulation of HCC development. Circular RNAs can target competitive adsorption to down-regulate the expression of corresponding miRNAs, relieve miRNA repression of downstream target genes, and at the same time participate in the development of HCC through multiple signaling pathways. [15]. Circβ-catenin, a recently discovered circRNA encoding a protein, can promote the growth of hepatocellular carcinoma cells by activating the Wnt pathway after translation. [16]. A study showed that [17] , a diagnostic

model composed of hsa_circ_0000976, hsa_circ_0007750, hsa_circ_0139897, was used to diagnose small hepatocellular carcinomas (<3 cm in diameter), with an AUC value of 0.862, sensitivity of 81.5%, specificity of 91.0%, and a significantly higher sensitivity than that of AFP, which indicates that CircRNA can be used as an early liver cancer diagnostic tool. CircRNA has potential value as a diagnosis of early hepatocellular carcinoma. However, the core issues of CircRNA generation mechanism, precise intracellular localization and degradation pathway have not been fully elucidated yet. In order to realize the application of CircRNA in the early clinical diagnosis and treatment of HCC, a series of studies are urgently needed to detect the level of CircRNA in body fluids and evaluate its value as a biomarker.

4.3. DNA Methylation

DNA methylation is an important epigenetic modification process, the essence of which is the addition of methyl groups to specific sequences of genes under the action of DNA methyltransferases, using S-adenosylmethionine as a methyl donor. This modification mechanism plays a key role in maintaining normal physiological functions of cells, regulating embryonic development, and transmission of genetic information. Recent studies have shown that aberrant changes in DNA methylation patterns are closely related to tumor development, and are now applied in the early diagnosis of HCC. Earlier studies reported that methylation of tumor suppressor genes RASSF1A, p16 and p15 are potential serum markers for early diagnosis of hepatocellular carcinoma [18] . Another study [19] showed that the AUC value of the combination of six DNA methylation markers, HOXA1, EMX1, AK055957, ECE1, PFKP and CLEC11A, for the diagnosis of early-stage HCC was 0.96, with 95% sensitivity and 92% specificity, and the diagnostic value of the DNA methylation markers was significantly better than that of AFP in this study. However, there is still a lack of clinical evidence from large-sample, multicenter and prospective studies on DNA methylation, and a large number of clinical trials are needed to confirm whether it can be used in the clinic.

5. Conclusion

China is a country with a high incidence of hepatocellular carcinoma. The incidence rate is increasing. Screening and early diagnosis and treatment are generally recognized worldwide as an effective means to reduce the incidence and mortality of hepatocellular carcinoma. However, the incidence rate and mortality rate of hepatocellular carcinoma are close to those of China, indicating that the early diagnosis rate of hepatocellular carcinoma is still low. Early diagnosis of hepatocellular carcinoma has become a hotspot and a challenge in clinical and basic research of hepatocellular carcinoma. Most of the tumor markers mentioned in the paper are limited to laboratory studies, and the reliability and clinical applicability of their results still need to be further verified. For the early detection of hepatocellular carcinoma, although the strategy of combining multiple tumor markers can effectively make up for the limitations of single-marker detection and improve the diagnostic accuracy, the increase of testing items inevitably increases the economic burden of patients. The combination of multiple markers and the standardization of marker detection methods are expected to increase the detection rate of early liver cancer patients and improve the overall prognosis of liver cancer patients.

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