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Review

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Tumor Markers and Hepatocellular Carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) cancer is a lethal cancer and early diagnosis of HCC is very important for improving the survival rate of patients. Measurement of Alfa-Fetoprotein (AFP) in combination with iconography and liver biopsy are commonly used in the diagnosis of liver cancer as well as HCC. Despite the wide use of AFP in HCC diagnosis and introducing this tumor marker as the gold standard biomarker for HCC detection, the specificity and sensitivity of AFP used in screening for HCC is very controversial. In recent years, along with development of molecular biology and extensive research about ethnology of cancers and simultaneously advances in technology, researchers identified new tumor markers in this disease. In the latest researches various biomarkers including embryonic antigen, protein antigens, cytokines, enzymes and isoenzymes and related genes for effective early diagnosis and monitoring of hepatocellular carcinoma are introduced. In this review, a summary of the data of various studies that discuss new tumor markers involved in hepatocellular carcinoma and classification of these biomarkers was investigated.

Key words: Tumor markers, hepatocellular carcinoma, liver disease

1. INTRODUCTION

Hepatocellular carcinoma (HCC) is a major health problem around the world and the fifth cause of cancer-related deaths (1, 2). According to the World Health Organization (WHO) reports, HCC involved about one million people annually. Hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection in 70–95% of HCC patients and chronic necro-inflammatory hepatic disease (cirrhosis liver) in 60–80% of patient are the most important Hepatocellular Carcinoma (HCC) risk factors (3, 4). HCC is mostly diagnosed after the disease progresses, when survival rates are low. Diagnosis of HCC is commonly done using the serum marker Alfa-Fetoprotein (AFP) in combination with ultrasonography. Various other serologic markers are being tested to help improve the accuracy of the diagnosis. Then, when the diagnosis of HCC remains unclear, confirmation by liver biopsy can be performed. One of the most important issues for improving the survival rate for HCC patients is early diagnosis. Sensitivity and specificity of serum level of HCC biomarkers and their clinical application have been summarized in

Table 1 (5).

Table 1. Serum level of biomarkers for HCC (sensitivity, specificity and clinical application)

Biomarker	Serum level	Sensitivity%	Specificity%	Clinical Application
Embryonic Antigen				
AFP	Up regulation	41.0-65.0%	80.0-94.0%	Early diagnosis
GPC_3	Up regulation	77%	96%	Early diagnosis
Protein antigen				
HSP	Up regulation	57.5 %	85.0 %	Prognosis
SCCA	Up regulation	84%	46%	Early diagnosis
GP_{73}	Up regulation	76.9%	-	Diagnosis
Enzyme and isoenzymes				
DCP	Up regulation	-	-	-
GGT	Up regulation	43.8%	-	Diagnosis
AFU	Up regulation	90%	97.5%	Diagnosis
$hCBR_2$	-	-	-	-
$GOLPH_2/GP_{73}$/GOLM1	-	-	-	-
Cytokine				
TGF-β1	Up regulation	89.5 %	94%	Prognosis
VEGF	Up regulation	-	-	Recurrence and prognosis
Growth factors and their receptors				
TSGF	Up regulation	82%	-	-
EGFR	-	-	-	-
FGF	-	-	-	-
Molecular biomarker				
AFP mRNA	Upregulation	-	-	Recurrence and prognosis
GGT mRNA	Upregulation	-	-	-
IGF-II mRNA	Upregulation	-	-	-
Albumin MRNA	Upregulation	-	-	-
miR-21	Upregulation	87.3%	92%	Diagnosis
miR-500	Downregulation	-	-	Prognosis
miR-122	Downregulation	-	-	Prognosis
miR-29	Downregulation	-	-	Prognosis

HCC, hepatocellular carcinoma; AFP, α-fetoprotein; HSP, heat shock proteins; GPC3, Glypican-3; SCCA, squamous cell carcinoma antigen; GGT; γ-glutamyl transferase; AFU, α-l-fucosidase; TGF-β1, transforming growth factor-β1; VEGF, endothelial growth factor; miR, miRNA. Tumor markers are measurable biochemical glycoproteins that have been associated with malignant tumor. Tumor biomarkers are made by the body in response to neoplasm conditions. These soluble molecules can be found in the blood or urine when cancer is present. Most tumor markers are proteins and glycoproteins and can serve as a useful measurement tool to diagnose various types of cancer as

well as to guide more specific treatment (6). Researches are continuously checking the clinical significance of tumor markers. Multitude of diseases, mainly various types of cancer, can be detected better by the use of tumor biomarkers. Hepatocellular carcinoma (HCC) is one such cancer that can use diagnostic, therapeutic, and prognostic tumor markers (7). Findings of a previous study suggested that to prolong the lifetime of (HCC) patients, early diagnosis of HCC and effective treatment is crucial. According to these findings, showing new high sensitivity and specificity tumor markers for HCC are one of the important challenges (8). In this paper, a summary of the main data available from published papers about new tumor markers involved in hepatocellular carcinoma was investigated. Classification of HCC tumor markers are schematically shown in Figure 1.

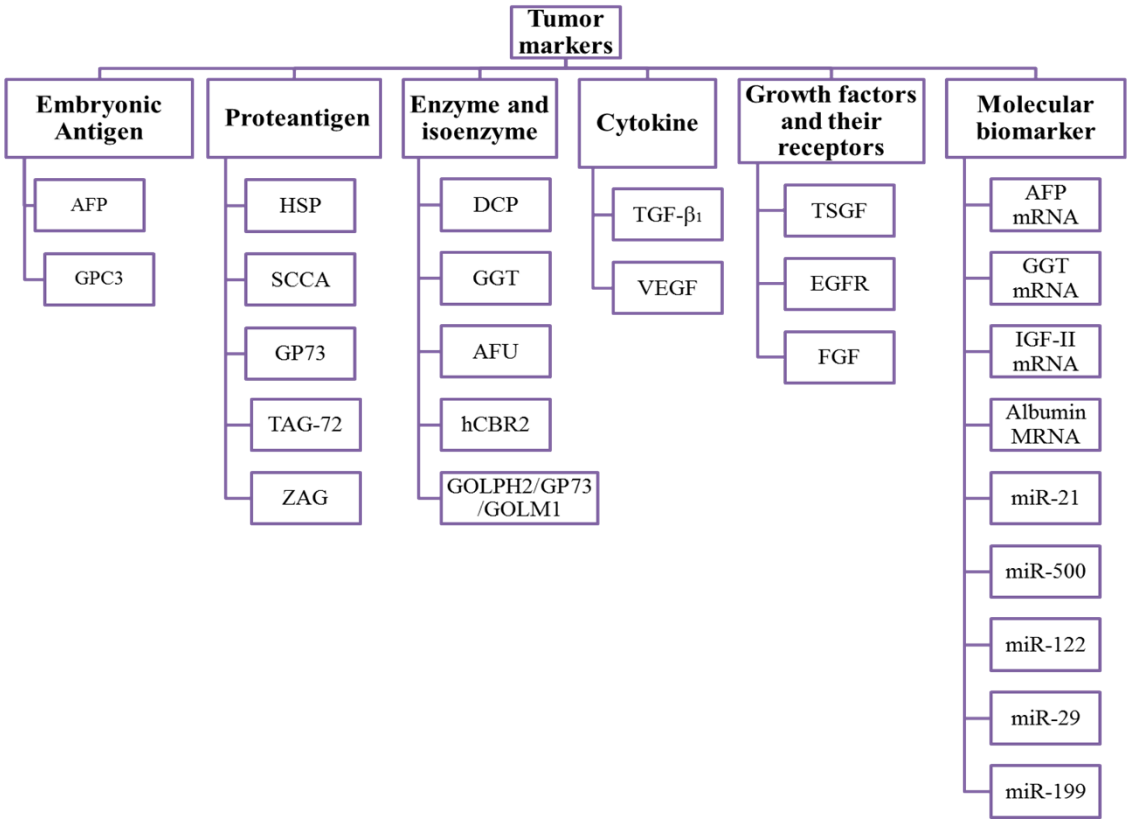

Figure 1. Hepatocellular carcinoma tumor markers

2. Embryonic Antigen

2.1. Alpha-Fetoprotein (AFP)

The first serologic test for detection and clinical follow-up of patients with hepatocellular carcinoma was alpha-fetoprotein (AFP) which has been considered as the gold standard tumor marker for HCC (9). AFP is a glycoprotein produced by the fetal liver and yolk sac during pregnancy. AFP Serum levels are significantly increased in HCC, but this is not often the case (10). Additionally, AFP elevation has also been demonstrated in patient with the acute and chronic viral hepatitis (HBV) and HCV as well as in patients with chronic necro-inflammatory hepatic diseases such as cirrhosis. Since AFP increase occurs in many diseases, it is necessary to assess diagnostic value of serum concentrations of AFP. Consistently elevated serum AFP levels greater than 400 ng/mL are indicative of HCC. Lower serum concentrations often exist temporarily in benign liver disease. If a patient has known risk factors for HCC, such as the cirrhosis, increasing levels of AFP have been demonstrated to correlate with the progression of HCC (11). It should be noted that, AFP serum concentrations do not correlate with the prognostic values of HCC such as stage, tumor size or disease progression and ethnicity. In addition, in some cases of HCC, increase of AFP is not clear at all. Findings of a previous clinical study indicated that serum AFP had a sensitivity of 41-65% and specificity of 80-94% when the cut-off value is 20 ng/ml (12). Development and applications of biological chemistry have determined that total AFP can be divided into three different glycoforms (AFP-L1, AFP-L2 and AFP-L3 according to their binding ability to the lectin lens agglutinin (LCA). High percentage of AFP-L3 has been demonstrated to be associated with pathologically malignant characteristics, worse liver function, larger tumor mass and poor survival in HCC patient (13). AFP-L1 is non-LCA-bound and exists in various benign liver diseases such as cirrhosis. AFP-L3 is an LCA-bound that is detected in the serum of patients with HCC at a cut-off value of 15%. The serum level of AFP-L3 has a sensitivity and specificity of 96.9 and 92%, respectively, in diagnosis of HCC (14). AFP-L3, as compared with AFP, has better sensitivity and specificity for the early diagnosis of HCC. Leerapun A et al. have demonstrated that the diagnostic specificity of AFP-L3 for early diagnosis of HCC reaches

100% when HCC patients have a cut-off value of AFP-L3 35% and serum AFP 10-200 ng/ml (15). In the early diagnosis of HCC at stage I or when the tumor size was <2 cm, AFP-L3 that was measured by using the μTAS method, was showed with high sensitivity (42.5 and 46.0%, respectively). Furthermore, Kobayashi M et al. have indicated that when the cut-off value of AFP-L3 is 5%, sensitivity for HCC reaches 47.2% compared with AFP that is 38% (16). In general, it can be concluded from the research results that AFP-L3 seems to be a useful marker for early diagnosis of HCC compared with AFP alone.

2.2. Glypican-3 (GPC3)

GPC3 is a membrane-anchored heparin sulfate proteoglycan that is linked to the cell membrane by a glycosyl-phosphatidylinositol (GPI) anchor (17) .GPC3 has been shown to interact with growth factors and modulate their activities. In addition, GPC3 is involved in the cellular process of regulating cell growth, development, differentiation and migration. Soluble GPC3, which lacks GPI, controls the growth of HCC by removing several growth factors such as Wnts, hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) from the surface of HCC cells in vitro and in vivo (18). GPC3 promotes the growth of HCC by stimulating Wnt signaling (19).expression of GPC3 (at both mRNA and protein levels) in the serum of HCC patients was higher than that in the serum of healthy adults or patients with benign disease. Furthermore, correlation between GPC3 expression (at both mRNA and protein levels) and tumor stage, size and AFP level has not been reported. Shirakawa H et al. indicated that serum level of GPC3 had a sensitivity of 77% and specificity of 96% (20). Also, the previous researches have demonstrated that GPC3 is found in 40–53% of HCC patients and 33% of HCC patients seronegative for both AFP and Des-gamma carboxyprothrombin (DCP) (21, 22). Recently, Shirakawa et al. have shown that survival rate between the GPC3-positive and GPC3-negative HCC patients was different. Patient with GPC3 positivity were associated with poor prognosis. So it can be said GPC3 has been indicated to be an independent prognostic factor for the overall survival (23). According to results from recent research GPC3 is a potential marker for HCC diagnosis.

3. Proteantigen

3.1. Heat shock protein (HSP)

Heat shock protein (HSP) is a highly conserved stress response protein that is expressed under stress conditions, including carcinogenesis and inflammation (24, 25). HSP is also a group of proteins that play a major role in the folding of cellular proteins (26). Furthermore, HSPs promote carcinogenesis through inhibiting the pathways of tumor suppression, in more advanced stage (27). Especially Hsp27 and Hsp70 have showed stronger features of carcinogenesis by inhibiting apoptosis and senescence and can cause treatment-resistant and facilitate metastasis. Luk, J et al. have demonstrated overexpression of HSP70 and HSP27 in HCC tissues and in the microenvironment of HCC that can increase tumor growth and metastasis (28). However, HSP70 induced tumor cell growth by stabilizing cyclin D1 and inhibited the apoptosis of tumor cells by suppressing the p53 signaling pathway (29). The stained intensity of HSP70 was positively associated with tumor size and tumor stage. In addition HSP27expression only correlated with HCC infected by hepatitis B virus (HBV). The sensitivity and specificity of HSP70 in diagnosis of of HCC were found to be 57.5 and 85%, respectively (30). In another research Shin E et al. have shown HSP70 expression is detected in 282 of 392 HCC cases (71.9%), while only 14 of 115 non-neoplastic liver tissues expressed HSP70 ($P<0.001$) (5). Overall, HSP70 and HSP27 are potential bio markers for HCC. Furthermore, HSP70 can be an indicator of poor prognosis for HCC.

3.2. Squamous cell carcinoma antigen (SCCA)

SCCA is a serine protease inhibitor expressed in epithelial tumors and protects tumor cells from apoptosis. Guido M et al. showed that the expression of SCCA increased in the early stages of HCC (31). The sensitivity and specificity of SCCA in detecting HCC were found to be 84**%** and 46% respectively (32). Zhou, L et al. have reported that SCCA is an important diagnostic marker's supplement for the diagnosis of HCC because SCCA, unlike AFP, has high sensitivity and low specificity (33). In another study Beneduce, L et al. have suggested that SCCA-IgM immune complex, a circulating immune complex composed of SCCA and IgM, is detectable in the sera of chronic hepatitis, cirrhosis and HCC compared with the healthy control population. (34). In previous studies the results have shown that SCCA-IgM IC can be a novel potential serum marker for HCC and combination of SCCA-IgM IC and AFP can improve the diagnostic rate of HCC.

3.3. Golgi protein 73 (GP73)

GP73 (also known as Golph2 and GOLM1) is a protein that is considered as a member of the type II Golgi-specific membrane protein (35). GP73 is overexpression in various types of cancer. Ito Y et al. have demonstrated that the serum GP73 is increased in primary hepatic carcinoma (PHC and is intimately correlated with liver diseases, especially HCC) and GP73 expression can be a potential factor in many hepatic diseases (36). On the other hand, Riener M et al. have reported that GP73 is expressed by normal biliary epithelial cells and hepatocytes do not express GP73 and it can have higher specificity of HCC diagnosis (37). Zhou Y et al. have investigated the sensitivity of HCC diagnosis compared with AFP and their results have showed that sensitivity of GP73 was 76.9% that was significantly increased compared with AFP (48.6%), suggesting GP73 is effective serum biomarker in detecting of HCC (38, 39). In general, it is important that correlation between GP73 and tumor size, stage,

recurrence and prognosis of HCC remains to be clarified, Thus, role of GP73 in the diagnosis remains to be extensively investigated (38-40).

3.4. Tumor-associated glycoprotein 72 (TAG-72)

TAG-72 is a glycoprotein complex that is found on the surface of cancer cells and overexpressed in the majority of human adenocarcinoma including gastric, colon and pancreatic cancer whereas it is lightly expressed in normal tissue (41). In recent study, Milenic, D.E et al. have reported that the expression of TAG-72 is increased in HCC tissues compared with normal liver tissues and over expression of TAG-72 is associated with promoting of tumor invasion and metastasis and poor survival. Therefore TAG-72 can be a potential prognostic marker for HCC, (42). Also, Louhimo J et al. have found that anti-TAG-72 monoclonal antibody can be used for the clinical detection of tumors and can be measured with radioimmunoassay. Furthermore this tumor marker has an appropriate specificity for identification of relapses of gastric cancer and in the follow up of the treatment (43, 44). On the other hand Ucar, E et al. have proven that TAG-72 is the target of the anti-cancer drugs anatumomab mafenatox and minretumomab (45). Thus, based on the results of previous research it can be concluded TAG-72 is a potential prognostic marker for HCC and this has various clinical implications in both diagnosis and treatment of cancers, particularly HCC.

3.5. Zinc-α2-glycoprotein (ZAG)

ZAG is a member of the class I major histocompatibility complex (MHC-I) family and may have a role in the induction of the immune response (46). This protein plays many important functions in the human body including fertilization and lipid mobilization and several unrelated functions such as RNase activity, regulation of melanin production, hindering tumor proliferation, and transporting of nephritic by-products but still, it is considered as a protein with an unknown function (46, 47). Wang, F et al. have demonstrated that ZAG is a soluble glycoprotein and up regulated in several types of cancer such as breast, lung and prostate cancers and HCC and it can be considered as a novel candidate biomarker for these cancer types (48); But it can be said, the function of the ZAG under physiologic and cancerous conditions, especially HCC, needs to be investigation more.

4. Enzymes and Isozymes

4.1. Des-γ-carboxyprothrombin (DCP)

DCP is an abnormal protein produced by the malignant hepatocyte. The expression of DCP induced by the absence of vitamin K. In fact, it is the process of malignant transformation in the liver cells associated with insufficient reaction of γ-glutamyl carboxylation that causes the production of DCP. Extensive researches by Marrero, J.A et al. have showed that the level of DCP in the serum of patients with HCC is higher than that in patients with chronic hepatitis and cirrhosis. The serum level 0.1 AU/mL (100 ng/mL) on ELISA is considered in HCC or tumor recurrence and finally study results concluded that a DCP value of 125 mAU/mL is the best sensitivity and specificity for distinguishing patients with HCC from those with cirrhosis (49). On the other hand, Mracek T et al. have reported that normal level of DCP is correlated well with successful tumor resection and it appears that DCP can be a potential marker of tumor activity in HCC (50). Also, recent studies that were conducted by Zhao, Y J proved that the combined detection of DCP and AFP can improve the diagnostic sensitivity and can be used to predict recurrence of HCC (5). Eventually, it is important to note that Yamamoto K et al. have been concluded that the level of DCP is profoundly associated with a larger tumor and vascular invasion and serves as a more specific tumor biomarker compared with AFP and AFP-L3 (51).

4.2. γ-glutamyl transferase (GGT)

GGT is a plasma membrane-bound enzyme that is synthesized in the microtomes of human cells (52, 53). The GGT expression is increased in embryo livers and is decreased after birth; The GGT assay is used to help detect liver disease and bile duct obstructions (54). It is usually recommended along with other liver tests such as ALT, AST, ALP, and bilirubin. In general, an increased GGT level indicates the dysfunction of liver. Yao D.F et al. have reported that serum gamma-glutamyl transferase (GGT) in healthy adults was definitely produced by hepatic Kupffer cell and endothelial cell of bile duct (55). In another study, Cui R et al. have showed that cholestasis, inflammation and development of HCC can increase the level of serum GGT to a moderate or high degree, thus GGT may serve as a potential biomarker for HCC (56). The sensitivity of GGT to detect small HCC is 43.8%. Study results by Wang C S et al. have demonstrated that determination of GGT, DCP and AFP simultaneously can improve the accuracy rate of diagnosis of HCC (56, 57).

4.3. α-l-fucosidase (AFU)

AFU is a lysosomal enzyme found in human cells, blood and body fluid, with a function to hydrolyze fucose glycosidic linkages of glycoprotein and glycolipids (58). AFU activity in the serum of healthy adults is low whereas AFU activity in the serum of HCC patients increases (59). Montaser, M. et al. have reported that the sensitivity, specificity and diagnostic accuracy of AFU at the cut-off value of 2.3005 μmol/l/min were 90, 97.5 and 94.9%, respectively. In general, from results of various studies it can be concluded that AFU measurement is useful in association with AFP. Also, the combined detection of AFP and AFU can be useful in early diagnosis of HCC and can serve as an excellent supplementary to AFP. However, the clinical value of AFU remains to be clarified because of the poor specificity of AFU and overexpression in diabetes, pancreatitis and hypothyroidism patients (60, 61).

4.4. Human Carbonyl Reductase 2

HCR2 is expressed in the human liver and kidney and its main function is detoxification of the reactive alpha-dicarbonyl compounds and reactivating oxygen species deriving from oxidative stress. Liu S et al. have reported that the human carbonyl reductase 2 levels are inversely associated with the pathological grading of HCC (62). However more researches are needed to determine the role of this enzyme in HCC.

4.5. Golgi Phosphoprotein 2

(GOLPH2/GP73/GOLM1), a type-II Golgi transmembrane protein, is up-regulated in various cancer types. Its Golgi luminal domain is the major functional domain (GOLPH2). Marrero, J.A et al. have shown higher sensitivity of GOLPH2 than AFP in the diagnosis of HCC (63). A recent study conducted by Riener, M.O et al. has demonstrated that GOLPH2 protein was significantly expressed in tissues of HCC (71%) and bile duct carcinoma (85%) patients (37). According to the results this enzyme can be new tumor marker for detecting of HCC is considerable.

5. Cytokines

5.1. Transforming growth factor-β1 (TGF-β1)

TGF-β1 is a pluripotent growth factor belonging to a superfamily of polypeptide signaling molecules involved in the regulation of cell proliferation, differentiation, embryo formation, angiogenesis, and immune functions (64-66)**.** It is significantly produced in tumor cells, inhibits the proliferation of tumor specific cytotoxic T lymphocytes (CTL) and NK cells and stimulates the growth of tumor cells (67). Giannelli G et al. have reported that the serum level of TGF-β1 increased in HCC patients compared with healthy adults or patients with non-malignant liver disease TGF-β1 and TGF-β1 mRNA may serve as sensitive indicators to detect HCC which is induced by HBV, with the sensitivity and specificity of 89.5 and 94.0% respectively; also the polymorphism of TGF-β1 expression can improve susceptibility to tumor formation (68). In recent years researchers have considered TGF-β1 signaling pathway as a tumor therapeutic target (69). Dituri F et al. have reported that TGF-β1 is inversely associated with E-cadherin but correlated with VEGF in HCC (70). In general, according to the role of TGF- β1 in cancer cell microenvironment, angiogenesis and cell proliferation it can be said that this cytokine can be considered in next researches.

5.2. Vascular endothelial growth factor (VEGF)

VEGF is a signal protein expressed by cells that stimulates vasculogenesis and angiogenesis. In addition vascular endothelial growth factor (VEGF) is has a special role in angiogenesis by promoting new vessel formation and inducing tumor invasion and metastasis. Also overexpression of VEGF has been shown in various malignancy condition (71). Xiang Z. L. et al. have reported that expression of VEGF is strongly high in HCC patients vs. healthy people. Furthermore, expression of VEGF is correlated with tumor recurrence and poor prognosis and survival. Thus, the overexpression of VEGF can serve as a biologic tumor marker in predicting poor prognosis and survival in HCC patients (72).

6. Growth Factors and Their Receptors

6.1. Tumor- Specific Growth Factor (T S G F)

Tumor specific growth factor (TSGF) is a factor that helps in inducing the growth of tumor blood vessels similar to VEGF. Liang YR, et al. have demonstrated that TSGF significantly correlates with hyperplasia of tumor tissue and microenvironment capillary vessels whereas similar correlation has not been shown between TSGF and hyperplasia of non-tumor blood vessels (73). Researches extensively indicated the high sensitivity of TSGF for detection of neoplasm condition. TSGF secretes into peripheral blood by malignant tumors during their growing period (74). Lawicki S et al. have reported that serum levels of TSGF can indicate the existence of tumor, therefore, TSGF can be used as a diagnostic marker in diagnosis of various cancer type such as HCC (75). In another research KornW.M. showed that the sensitivity of TSGF at the cut-off value of 62 U/mL was 82%. The simultaneous determination of TSGF (at the cut-off value of 65 U/mL), AFP (at the cut-off value of 25 ng/mL), and serum ferritin (at the cut-off value of 240 ng/mL) can provide a sensitivity and specificity of 98.4% and 99%, respectively for detecting of HCC (76). Finally, it can be concluded from previous studies that TSGF along with other markers such as AFP and ferritin may show better sensitivity and specificity for diagnosis of HCC.

6.2. Epidermal Growth Factor Receptor Family (EGFR)

EGFR is the cell-surface receptor tyrosine kinases, a members of the epidermal growth factor family (EGF-family), that has a major role in regulating cell proliferation and consists of four related transmembrane tyrosine kinase receptors: EGFR (erbB-1), c-erb-2 (Her-2/neu), c-erb-3 (HER-3), and c-erb-4 (HER-4) (77). Osada S et al. have indicated that high levels of EGFR expression are closely correlated with early recurrence and poor prognosis following resection of hepatocellular carcinoma. On the other hand, also hepatocyte growth factor/scatter factor (HGF/SF) is a growth factor that induces embryonic development and liver regeneration. Mechanisms of hepatocarcinogenesis of HGF are via paracrine system that induces its cellular receptor, c-met and high c-met expression. Osada S et al. also reported that HGF/SF is involved in invasive-type HCC and is associated with metastasis (78).

6.3. Fibroblast Growth Factor (FGF)

Fibroblast growth factors (FGFs) and their receptors play essential roles in regulate many function of cell such as

regulating cellular proliferation, survival, migration and differentiation. There is extensive evidence for the importance of FGF signaling in the pathogenesis of various type of malignancy. On the other hand, the result of current researches showed that FGF is a heparin-binding polypeptide and can induce an especial mitogenic effect on endothelial cells. Poon, R.T.-P., et al. have demonstrated that serum levels above the median of >10.8 pg/mL can decrease disease-free survival in HCC patients (79). Also another study conducted by Day, A.A et al. have reported that targeted therapy lenalidomide that inhibits fibroblast growth factor (FGF) is useful for HCC patients (79, 80).

7. Molecular Biomarkers

7.1. Circulating Nucleic Acids: mRNAs

The analysis of circulating nucleic acids in plasma is suggested for noninvasive monitoring of a variety of physiological and pathologic conditions and it provide a valuable approach for development of pathology-related markers (81-83).

7.2. AFP mRNA

AFP mRNA is a potential bio marker for active HCC cell. AFP mRNA could be used as a significant marker for spreading of HCC in blood. Chan, A.K., et al. have reported that in liver cancer, AFP mRNA expression is higher than healthy control (84). Since AFP is a gold standard tumor marker for detection of HCC, it can be concluded AFP mRNA effectively predicts tumor recurrence and metastasis following surgery of HCC patients.

7.3. Gamma - Glutamyl Transferase mRNA (GGT mRNA)

Sheen, I.S et al. have indicated that GGT mRNA can be found in the serum and liver tissues of healthy adults, patients with liver disease, benign liver tumor, HCC, and secondary tumors of the liver. Presence of type B GGT mRNA in cancerous tissue was significantly associated with high serum level of AFP and poor survival in HCC (33, 85).

7.4. Insulin-Like Growth Factor II (IGF-II) mRNA

Expression of IGF-II mRNA can be a valuable tumor marker for diagnosis, metastasis, and following postoperative recurrence in HCC. Himoto T et al. have reported that the assay of serum insulin-like growth factor-II (IGF-II) (at the cut-off value of 4.1 mg/g, prealbumin) has sensitivity of 63%, specificity of 90%, and accuracy of 70% in the detection of HCC patients. In another research Tsai J et al. showed that IGF-II's m-RNA is a supplementary tumor biomarker to AFP for detecting of HCC. The simultaneous assay of IGF-II m-RNA and AFP (at the cutoff value of 50 ng/mL) can improve the sensitivity to 80% and accuracy to 88% (86, 87).

7.5. Albumin mRNA

Albumin is a family of globular proteins; Albumin is a soluble protein and is commonly found in blood and is synthesized by the liver. Other blood proteins are different from Albumin from in that they are not glycosylated. The albumin's mRNA is in human blood and could be a clinically potential biomarker for liver pathologies. Cheung, S.T. et al. have demonstrated that high serum albumin's mRNA level predicted the 2-year recurrence rate with sensitivity and specificity of 73% and 70%, respectively (88).

7.6. MicroRNAs (mi RNAs)

MicroRNAs (miRNAs) are small (21-23 nucleotides), noncoding functional RNAs. HCC associated miRNAs could serve as diagnostic and prognostic HCC biomarkers. miRNAs can be used as accurate indicators for predicting metastatic condition or survival in HCC patients. Function of MicroRNAs is to regulate gene expression by binding to specific messenger RNAs (mRNA) and inhibit their translation into protein. Furthermore, each type of miRNA can simultaneously downregulate hundreds of genes. In general, miRNA profiling has emerged as a valuable approach for phenotyping tumors (89, 90). miRNA assay has several advantages in compared with conventional gene expression profiling (in which protein-coding, messenger RNAs) including: stability of miRNAs, formalin-fixed samples (rather than frozen tissue) and the investigation of hundreds of miRNAs. Many independent groups have done comprehensive analyses of miRNAs in HCC, and a plethora of information on miRNA markers exist. Studies have shown many of miRNA signatures in HCC associated with important parameters, such as metastasis differentiation, HBV or HCV infection, tumor recurrence, and patient survival (90, 91). Furthermore miRNAs promote HCC carcinogenesis by inducing cancer stem cell and by controlling cell proliferation and apoptosis and some of miRNA correlated to HCC progression by controlling cell migration and invasion. These HCC-associated miRNAs undoubtedly provide new insights into the molecular basis. Hou, J et al. have demonstrated that a molecular biomarker for HCC is miR-500 (miRNA) that is highly expressed in embryo liver, downregulated in the process of liver development and then upregulated in the process of cirrhosis. Also in another research done by the same team it has been reported that miR-29 and miR-199 are downregulated in HCC cells, suggesting its role as a potential prognostic marker for therapy and follow up of HCC patient (92). Xu Y et al. have showed that miR-122 is a liver-specific microRNA that is downregulating in HCC. The loss of miR-122 induces HCC cell migration and invasion and previous study done by Xu, Y., et al. analyzed the miRNA signatures of a large number of tumor samples. It has been shown that only miR-21 is upregulated in the tumors. Also new researches have reported that miR-21 is a central oncomiR (93, 94). Tomimaru Y et al. demonstrated that serum miR-21 level in patients with HCC was significantly high in compared with patients with chronic hepatitis and

healthy people. ROC analysis showed that differentiation of HCC patients from healthy adults is possible with the sensitivity and specificity 87.3 and 92%, respectively, therefore, it can be said miR-21 is a promising genetic biomarker of HCC diagnosis (95). Cancer and malignancy is a complex disease and various factors are involved in its development. Tumor markers are glycoproteins and their expression level is increased during tumor progression. HCC is not an exception. HCC Patient show increasing tumor markers that are closely associated with the occurrence, development, recurrence and poor prognosis of disease (96). Overall, according to results of all studies about roles of mi-RNAs in diagnosis of various type of cancer particularly HCC, it can be concluded that mi-RNAs assay along with other tumor markers help in improvement of the diagnosis of HCC, especially in metastatic conditions.

8. CONCLUSION

Most HCC biomarkers exist in the clinical setting however, a single test that has high accuracy and specificity for detection of early stage of HCC, has not been introduced yet. Therefore, sensitive test strategies should be considered to improve the early stage diagnostic of HCC including: i) the combined detection of serum biomarkers that can supplement each other in order to accelerate the early diagnostic rate. The sensitivity of AFP-L3, FC-GP73, AFU and SCCA is better than that of AFP .Combined detection can improve the diagnosis of HCC and reduce misdiagnosis. The GPC3 and TGF-β1 biomarkers have a very high specificity. Combined detection with AFP can excellently improve the ability of detection and diagnosis for HCC. ii) Select the appropriate tumor marker based on the stage of the disease: for example, AFP mRNA, TGF-β1 and VEGF should be superior options in the prediction of HCC metastasis. iii) Priority in the use of genetic markers which is likely to improve the early diagnostic of HCC, as well as predict the recurrence of tumors. The genetic markers are new perspectives on early diagnosis of various cancers especially HCC, furthermore, more studies in the field of molecular tumor markers is required in order to introduce novel genetic markers that result in improved early diagnosis of HCC.

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REFERENCES

1. Sherman M, Peltekian KM, Lee C. Screening for hepatocellular carcinoma in chronic carriers of hepatitis B virus: incidence and prevalence of hepatocellular carcinoma in a North American urban population. Hepatology. 1995;22(2):432-8.
2. Mittal S, El-Serag HB. Epidemiology of HCC: Consider the Population. Journal of clinical gastroenterology. 2013;47:S2.
3. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology. 2007;45(2):507-39.
4. Sangiovanni A, Del Ninno E, Fasani P, De Fazio C, Ronchi G, Romeo R, et al. Increased survival of cirrhotic patients with a hepatocellular carcinoma detected during surveillance. Gastroenterology. 2004;126(4):1005-14.
5. Zhao Y-J, Ju Q, Li G-C. Tumor markers for hepatocellular carcinoma (Review). Molecular and clinical oncology. 2013;1(4):593-8.
6. Malati T. Tumour markers: an overview. Indian Journal of Clinical Biochemistry. 2007;22(2):17-31.
7. Behne T, Copur MS. Biomarkers for hepatocellular carcinoma. International journal of hepatology. 2012;2012.
8. Trinchet J, Alperovitch A, Bedossa P, Degos F, Hainaut P, Beers B. [Epidemiology, prevention, screening and diagnosis of hepatocellular carcinoma]. Bulletin du cancer. 2009;96(1):35-43.
9. Alpert E, Feller ER. Alpha-fetoprotein (AFP) in benign liver disease. Evidence that normal liver regeneration does not induce AFP synthesis. Gastroenterology. 1978;74(5 Pt 1):856-8.
10. Chen D-S, Sung J, Sheu J, Lai M-Y, How S, Hsu H, et al. Serum a-fetoprotein in the early stage of human hepatocellular carcinoma. Gastroenterology. 1984;86.9:(1404)
11. Wu JT. Serum alpha-fetoprotein and its lectin reactivity in liver diseases: a review. Annals of Clinical & Laboratory Science. 1990;20(2):98-105.
12. Gonzalez SA, Keeffe EB. Diagnosis of hepatocellular carcinoma: role of tumor markers and liver biopsy. Clinics in liver disease. 2011;15(2):297-306.
13. Khien V, Mao H, Chinh T, Ha P, Bang M, Lac B, et al. Clinical evaluation of lentil lectin-reactive alpha-fetoprotein-L3 in histology-proven hepatocellular carcinoma. The International journal of biological markers. 2000;16(2):105-11.
14. Singhal A, Jayaraman M, Dhanasekaran DN, Kohli V. Molecular and serum markers in hepatocellular carcinoma: predictive tools for prognosis and recurrence. Critical reviews in oncology/hematology. 2012;82(2):116.40-
15. Leerapun A, Suravarapu SV, Bida JP, Clark RJ, Sanders EL, Mettler TA, et al. The utility of lens culinaris agglutinin-reactive α-fetoprotein in the diagnosis of hepatocellular carcinoma: evaluation in a United States referral population. Clinical Gastroenterology and Hepatology. 2007;5(3):394-402.
16. Kobayashi M, Hosaka T, Ikeda K, Seko Y, Kawamura Y, Sezaki H, et al. Highly sensitive AFP-L3% assay is useful for predicting recurrence of hepatocellular carcinoma after curative treatment pre-and postoperatively. Hepatology Research. 2011;41(11):1036-45.
17. Filmus J, Capurro M, Rast J. Glypicans. Genome biology. 2008;9(5):224.
18. Zittermann SI, Capurro MI, Shi W, Filmus J. Soluble glypican 3 inhibits the growth of hepatocellular carcinoma in vitro and in vivo. International Journal of Cancer. 2010;126(6):1291-301.
19. Capurro MI, Xiang Y-Y, Lobe C, Filmus J. Glypican-3 promotes the growth of hepatocellular carcinoma by stimulating canonical Wnt signaling. Cancer research. 2005;65(14):6245-54.
20. Shirakawa H, Kuronuma T, Nishimura Y, Hasebe T, Nakano M, Gotohda N, et al. Glypican-3 is a useful diagnostic marker for a component of hepatocellular carcinoma in human liver cancer. International journal of oncology. 2009;34(3):649-56.
21. Capurro M, Wanless IR, Sherman M, Deboer G, Shi W, Miyoshi E, et al. Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma. Gastroenterology. 2003;125(1):89-97.
22. Nakatsura T, Yoshitake Y, Senju S, Monji M, Komori H, Motomura Y, et al. Glypican-3, overexpressed specifically in human hepatocellular carcinoma, is a novel tumor marker. Biochemical and biophysical research communications. 2003;306(1):16-25.
23. Shirakawa H, Suzuki H, Shimomura M, Kojima M, Gotohda N, Takahashi S, et al. Glypican-3 expression is correlated with poor prognosis in hepatocellular carcinoma. Cancer science. 2009;100(8):1403-7.
24. Chai Y, Koppenhafer SL, Bonini NM, Paulson HL. Analysis of the role of heat shock protein (Hsp) molecular chaperones in polyglutamine disease. The Journal of neuroscience. 1999;19(23):10338-47.
25. Suto R, Srivastava PK. A mechanism for the specific immunogenicity of heat shock protein-chaperoned peptides. Science. 1995;269(5230):1585-8.
26. Kiang JG, Tsokos GC. Heat shock protein 70 kDa :molecular biology, biochemistry, and physiology. Pharmacology & therapeutics. 1998;80(2):183-201.
27. Melcher A, Todryk S, Hardwick N, Ford M, Jacobson M, Vile RG. Tumor immunogenicity is determined by the mechanism of cell death via induction of heat shock protein expression. Nature medicine. 1998;4(5):581-7.
28. Luk JM, Lam CT, Siu AF, Lam BY, Ng IO, Hu MY, et al. Proteomic profiling of hepatocellular carcinoma in Chinese cohort reveals heat-shock proteins (Hsp27, Hsp70, GRP78) up-regulation and their associated prognostic values. Proteomics. 2006;6(3):1049-57.
29. Diehl JA, Yang W, Rimerman RA, Xiao H, Emili A. Hsc70 regulates accumulation of cyclin D1 and cyclin D1-dependent protein kinase. Molecular and cellular biology. 2003;23(5):1764-74.
30. Tremosini S, Forner A, Boix L, Vilana R, Bianchi L, Reig M, et al. Prospective validation of an immunohistochemical panel (glypican 3, heat

shock protein 70 and glutamine synthetase) in liver biopsies for diagnosis of very early hepatocellular carcinoma. Gut.7-1481:(10)61;2012 .
31. Guido M, Roskams T, Pontisso P, Fassan M, Thung SN, Giacomelli L, et al. Squamous cell carcinoma antigen in human liver carcinogenesis. Journal of clinical pathology. 2008;61(4):445-7.
32. Giannelli G, Marinosci F, Sgarra C, Lupo L ,Dentico P, Antonaci S. Clinical role of tissue and serum levels of SCCA antigen in hepatocellular carcinoma. International journal of cancer. 2005;116(4):579-83.
33. Zhou L, Liu J, Luo F. Serum tumor markers for detection of hepatocellular carcinoma. World journal of gastroenterology: WJG. 2006;12(8):1175-81.
34. Beneduce L, Castaldi F, Marino M, Quarta S, Ruvoletto M, Benvegnù L, et al. Squamous cell carcinoma antigen-immunoglobulin M complexes as novel biomarkers for hepatocellular carcinoma. Cancer-2558:(12)103;2005 . .65
35. Mao Y, Yang H, Xu H, Lu X, Sang X, Du S, et al. Golgi protein 73 (GOLPH2) is a valuable serum marker for hepatocellular carcinoma. Gut. 2010;59(12):1687-93.
36. Ito Y, Takeda T, Sakon M, Tsujimoto M, Higashiyama S, Noda K, et al . Expression and clinical significance of erb-B receptor family in hepatocellular carcinoma. British journal of cancer. 2001;84(10):1377.
37. Riener MO, Stenner F, Liewen H, Soll C, Breitenstein S, Pestalozzi BC, et al. Golgi phosphoprotein 2 (GOLPH2) expression in liver tumors and its value as a serum marker in hepatocellular carcinomas. Hepatology. 2009;49(5):1602-9.
38. Zhou Y, Yin X, Ying J, Zhang B. Golgi protein 73 versus alpha-fetoprotein as a biomarker for hepatocellular carcinoma: a diagnostic meta-analysis. BMC cancer. 2012;12(1):17.
39. Mao Y, Yang H, Xu H, Sang X, Lu X, Yang Z, et al. [Significance of Golgi glycoprotein 73, a new tumor marker in diagnosis of hepatocellular carcinoma: a primary study]. Zhonghua yi xue za zhi. 2008;88(14):948-51.
40. Zhang Y, Deng Z-s, Liao M-m, Wang N, Zhang X-q, Yu H-y. Tumor associated glycoprotein-72 is a novel marker for poor survival in hepatocellular carcinoma. Pathology & Oncology Research. 2012;18(4):911-6.
41. Sheer DG, Schlom J, Cooper HL. Purification and composition of the human tumor-associated glycoprotein (TAG-72) defined by monoclonal antibodies CC49 and B72. 3. Cancer research. 1988;48(23):6811-8.
42. Milenic DE, Brady ED, Garmestani K, Albert PS, Abdulla A, Brechbiel MW. Improved efficacy of α-particle–targeted radiation therapy. Cancer. 2010;116(S4):1059-66.
43. Louhimo J, Alfthan H, Stenman U-H, Haglund C. Serum HCGβ and CA 72-4 are stronger prognostic factors than CEA, CA 19-9 and CA 242 in pancreatic cancer. Oncology. 2004;66(2):126-31.
44. Iles RK. Ectopic hCGβ expression by epithelial cancer: malignant behaviour, metastasis and inhibition of tumor cell apoptosis. Molecular and cellular endocrinology. 2007;260:264-70.
45. Ucar E, Semerci E, Ustun H, Yetim T, Huzmeli C, Gullu M. Prognostic value of preoperative CEA, CA 19-9, CA 72-4, and AFP levels in gastric cancer. Advances in therapy. 2008;25(10):1075-84.
46. Hassan MI, Waheed A, Yadav S, Singh TP, Ahmad F. Zinc α2-glycoprotein: a multidisciplinary protein. Molecular Cancer Research. 200.906-892:(6)6;8
47. Russell ST, Tisdale MJ. Antidiabetic properties of zinc-α2-glycoprotein in ob/ob mice. Endocrinology. 2010;151(3):948-57.
48. Wang F, Geng Y, Zhang WM, Geng X, editors. Identification of ZAG protein as a novel serologic biomarker candidate for liver cancer. Advanced Materials Research; 2012: Trans Tech Publ.
49. Marrero JA, Su GL, Wei W, Emick D, Conjeevaram HS, Fontana RJ, et al. Des-gamma carboxyprothrombin can differentiate hepatocellular carcinoma from nonmalignant chronic liver disease in American patients. Hepatology. 2003;37(5):1114-21.
50. Mracek T, Stephens N, Gao D, Bao Y, Ross J, Ryden M, et al. Enhanced ZAG production by subcutaneous adipose tissue is linked to weight loss in gastrointestinal cancer patients. British journal of cancer. 2011;104(3):441-7.
51. Yamamoto K, Imamura H, Matsuyama Y, Kume Y, Ikeda H, Norman GL, et al. AFP, AFP-L3, DCP, and GP73 as markers for monitoring treatment response and recurrence and as surrogate markers of clinicopathological variables of HCC. Journal of gastroenterology. 2010;45(12):1272-82.
52. Puukka K, Hietala J, Koivisto H, Anttila P, Bloigu R, Niemelä O. Additive effects of moderate drinking and obesity on serum γ-glutamyl transferase activity. The American journal of clinical nutrition. 2006;83(6):1351-4.
53. Kristenson H, Trell E. Indicators of Alcohol Consumption: Comparisons Between a Questionnaire (Mm-MAST), Interviews and Serum γ-Glutamyl Transferase (GGT) in a Health Survey of Middle-aged Males. British journal of addiction. 1.304-297:(3)77;982
54. Szasz G. A kinetic photometric method for serum γ-glutamyl transpeptidase. Clinical chemistry. 1969;15(2):124-36.
55. Yao D-F, Dong Z-Z, Yao M. Specific molecular markers in hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int.7-241:(3)6;2007 .
56. Cui R, He J, Zhang F, Wang B, Ding H, Shen H, et al. Diagnostic value of protein induced by vitamin K absence (PIVKAII) and hepatoma-specific band of serum gamma-glutamyl transferase (GGTII) as hepatocellular carcinoma markers complementary to α-fetoprotein. British journal of cancer. 2003;88(12):1878-82.
57. Wang C-S, Lin C-L, Lee H-C, Chen K-Y, Chiang M-F, Chen H-S, et al. Usefulness of serum des-gamma-carboxy prothrombin in detection of hepatocellular carcinoma. World journal of gastroenterology: WJG. 2005;11(39):6115-9.
58. Deugnier Y, David V, Brissot P, Mabo P, Delamaire D, Messner M, et al. Serum α-L-Fucosidase: A New Marker for the Diagnosis of Primary Hepatic Carcinoma? Hepatology. 1984;4(5):889-92.
59. Bukofzer S, Stass P, Kew M, De Beer M, Groeneveld H. Alpha-L-fucosidase as a serum marker of hepatocellular carcinoma in southern African blacks. British journal of cancer. 1989;59(3):417.
60. Montaser MF, Sakr MA, Khalifa MO. Alpha-L-fucosidase as a tumour marker of hepatocellular carcinoma. Arab Journal of Gastroenterology. 2012;13(1):9-13.
61. Zhang S-Y, Lin B-D, Li B-R. Evaluation of the diagnostic value of alpha-l-fucosidase, alpha-fetoprotein and thymidine kinase 1 with ROC and logistic regression for hepatocellular carcinoma. FEBS open bio. 2015;5:240-4.
62. Liu S, Ma L, Huang W, Shai Y, Ji X, Ding L, et al. Decreased expression of the human carbonyl reductase 2 gene HCR2 in hepatocellular carcinoma. Cellular and Molecular Biology Letters. 2006;11(2):230-41.
63. Marrero JA, Romano PR, Nikolaeva O, Steel L, Mehta A, Fimmel CJ, et al. GP73, a resident Golgi glycoprotein, is a novel serum marker for hepatocellular carcinoma. Journal of hepatology. 2005;43(6):1007-12.
64. Wikström P, Stattin P, Franck-Lissbrant I, Damber JE ,Bergh A. Transforming growth factor β1 is associated with angiogenesis, metastasis, and poor clinical outcome in prostate cancer. The Prostate. 1998;37(1):19-29.
65. Willis BC, Liebler JM, Luby-Phelps K, Nicholson AG, Crandall ED, du Bois RM, et al. Induction of epithelial-mesenchymal transition in alveolar epithelial cells by transforming growth factor-β1: potential role in idiopathic pulmonary fibrosis. The American journal of pathology. 2005;166(5):1321-32.
66. Worster AA, Nixon AJ, Brower-Toland BD ,Williams J. Effect of transforming growth factor β1 on chondrogenic differentiation of cultured equine mesenchymal stem cells. American journal of veterinary research. 2000;61(9):1003-10.
67. Giannelli G, Fransvea E, Marinosci F, Bergamini C, Colucci S ,Schiraldi O, et al. Transforming growth factor-β1 triggers hepatocellular carcinoma invasiveness via α3β1 integrin. The American journal of pathology. 2002;161(1):183-93.
68. Giannelli G, Bergamini C, Marinosci F, Fransvea E, Quaranta M, Lupo L, et al. Clinical role of MMP-2/TIMP-2 imbalance in hepatocellular carcinoma. International journal of cancer. 2002;97(4):425-31.
69. Balzarini P, Benetti A, Invernici G, Cristini S, Zicari S, Caruso A, et al. Transforming growth factor-beta1 induces microvascular abnormalities through a down-modulation of neural cell adhesion molecule in human hepatocellular carcinoma. Laboratory investigation. 2012;92(9):1297-309.
70. Dituri F, Serio G, Filannino D, Mascolo A, Sacco R, Villa E, et al. Circulating TGF-β1-related biomarkers in patients with hepatocellular carcinoma and their association with HCC staging scores. Cancer letters. 2014;353(2):264-71.
71. Duffy AM, Bouchier-Hayes DJ, Harmey JH. Vascular Endothelial Growth Factor (VEGF) and Its Role in Non-Endothelial Cells: Autocrine Signalling by VEGF. VEGF and Cancer. 2004:133-44.
72. Xiang Z-L, Zeng Z-C, Fan J, Tang Z-Y, Zeng H-Y, Gao D-M. Gene expression profiling of fixed tissues identified hypoxia-inducible factor-1α, VEGF, and matrix metalloproteinase-2 as biomarkers of lymph node metastasis in hepatocellular carcinoma. Clinical Cancer Research. 2011;17(16):5463-72.
73. Liang Y, Wan D, Chen G, Lu Z, Li Y, Lin Y, et al. [Detection of serum tumor supplied group of factors and its clinical significance]. Ai zheng= Aizheng= Chinese journal of cancer. 2002;21(11):1251-3.
74. Yu B, Xu P, Wang Q, Zhou H, Zhou H. Clinical value of tumour specific growth factor (TSGF) and carbohydrate antigen-125 (CA-125) in carcinoma of the endometrium. Journal of International Medical Research. 2009;37(3):878-83.
75. Ławicki S, Bedkowska G, Gacuta-Szumarska E, Czygier M, Szmitkowski M. [The plasma levels and diagnostic utility of stem cell factor in patients with endometrial cancer and myoma uteri]. Polski merkuriusz lekarski: organ Polskiego Towarzystwa Lekarskiego. 2009;26(156):609-15.
76. Korn WM. Moving toward an understanding of the metastatic process in hepatocellular carcinoma. World J Gastroenterol. 2001;7(6):777-8.
77. Wieduwilt M, Moasser M. The epidermal growth factor receptor family: biology driving targeted therapeutics. Cellular and Molecular Life Sciences. 2008;65(10):1566-84.
78. Osada S, Kanematsu M, Imai H, Goshima S. Clinical significance of serum HGF and c-Met expression in tumor tissue for evaluation of properties and treatment of hepatocellular carcinoma. Hepato-gastroenterology. 2007;55(82-83):544-9.
79. Poon RT-P, Ng IO-L, Lau C, Yu W-C, Fan S-T, Wong J. Correlation of serum basic fibroblast growth factor levels with clinicopathologic features and postoperative recurrence in hepatocellular carcinoma. The American journal of surgery. 2001;182(3):298-304.
80. Day AA, Course ASR, editors. Gastrointestinal Cancers Symposium. The Symposium on Clinical Interventional Oncology; 2010.
81. Tong Y-K, Lo YD. Diagnostic developments involving cell-free (circulating) nucleic acids. Clinica Chimica Acta. 2006;363(1):187-96.
82. Tsang JC, Dennis Lo Y. Circulating nucleic acids in plasma/serum. Pathology. 2007;39(2):197-207.
83. Gahan PB, Swaminathan R. Circulating nucleic acids in plasma and serum. Annals of the New York Academy of Sciences. 2008;1137(1):1-6.
84. Chan AK, Chiu RW, Lo YD. Cell-free nucleic acids in plasma, serum and urine: a new tool in molecular diagnosis. Annals of clinical biochemistry. 2003;40(2):122-30.
85. Sheen I-S, Jeng K-S, Tsai Y-C. Is the expression of gamma-glutamyl transpeptidase messenger RNA an indicator of biological behavior in recurrent hepatocellular carcinoma? World Journal of Gastroenterology. 2003;9(3):468-73.
86. Himoto T, Kuriyama S, Zhang J-Y, Chan EK, Kimura Y, Masaki T, et al. Analyses of autoantibodies against tumor-associated antigens in patients with hepatocellular carcinoma. International journal of oncology. 2005;27(4):1079-85.
87. Tsai J, Jeng J, Chuang L, You H, Wang L, Hsieh M ,et al. Serum insulin-like growth factor-II as a serologic marker of small hepatocellular carcinoma. Scandinavian journal of gastroenterology. 2005;40(1):68-75.
88. Cheung ST, Fan ST, Lee YT, Chow JP, Ng IO, Fong DY, et al. Albumin

mRNA in plasma predicts post-transplant recurrence of patients with hepatocellular carcinoma. Transplantation. 2008;85(1):81-7.
89. Ferracin M, Veronese A, Negrini M. Micromarkers: miRNAs in cancer diagnosis and prognosis. 2010.
90. Xiong Y, Fang JH, Yun JP, Yang J, Zhang Y, Jia WH, et al. Effects of MicroRNA-29 on apoptosis, tumorigenicity, and prognosis of hepatocellular carcinoma. Hepatology. 2010;51(3):836-45.
91. Zhang J, Yang Y, Yang T, Liu Y, Li A, Fu S, et al. microRNA-22, downregulated in hepatocellular carcinoma and correlated with prognosis, suppresses cell proliferation and tumourigenicity. British journal of cancer. 2010;103(8):1215-20.
92. Hou J, Lin L, Zhou W, Wang Z, Ding G, Dong Q, et al. Identification of miRNomes in human liver and hepatocellular carcinoma reveals miR-199a/b-3p as therapeutic target for hepatocellular carcinoma. Cancer cell. 2011;19(2):232-43.
93. Xu Y, Xia F, Ma L, Shan J, Shen J, Yang Z, et al. MicroRNA-122 sensitizes HCC cancer cells to adriamycin and vincristine through modulating expression of MDR and inducing cell cycle arrest. Cancer letters. 2011;310(2):160-9.
94. Medina PP, Nolde M, Slack FJ. OncomiR addiction in an in vivo model of microRNA-21-induced pre-B-cell lymphoma. Nature. 2010;467(7311):86-90.
95. Tomimaru Y, Eguchi H, Nagano H, Wada H, Kobayashi S, Marubashi S, et al. Circulating microRNA-21 as a novel biomarker for hepatocellular carcinoma. Journal of hepatology. 2012;56(1):167-75.
96. Xieraili M, Yasen M, Mogushi K, Obulhasim G, Mayinuer A, Aihara A, et al. Villin 1 is a predictive factor for the recurrence of high serum alpha-fetoprotein-associated hepatocellular carcinoma after hepatectomy. Cancer science. 2012;103(8):1493-501.