[Frontiers in Bioscience S2, 571-577, January 1, 2010]

Clinical biomarkers in hepatocellular carcinoma (HCC)

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1. ABSTRACT

The Authors make a review of the most important molecules used today for detecting and screening hepatocellular carcinoma, analyzing their known biological features, advantages and limits especially concerning differential diagnosis from cirrhosis and others diseases. AFP, AFP-L3, DCP, Alpha-1-fucosidase are analyzed and all the current knowledge reviewed, as well as focusing on possible future applications and results of combined determination (what about another word here: evaluation etc) in HCC. Additionally other more recent molecules showing promising future clinical application are cited.

2. INTRODUCTION

Hepatocellular carcinoma (HCC) is today the fifth cause of tumor related death worldwide, and this tendency is increasing primarily due to HCV epidemic diffusion(1). Unfortunately the results from surveillance programs, even by combining serum marker control with US-study of the liver, are strongly influenced by the poor performance in terms of sensitivity and specificity of these markers(2).

Over the last 20 years the enormous development of biochemistry and laboratory technology has allowed an

Table 1. Summary of biomarker features for hepatocellular carcinoma.

Biomarker	Biologic Features	Cut-off	Sensitivity	Specificity
AFP	Fetal specific glycoprotein	200 ng/ml	40.8%	100%*
AFP-L3	Glycoprotein LCA-reactive	(15%)	75-96%	>95%
DCP	Protein induced by Vit. K absence	125 mAU/ml	89%	86.7%
Alpha-1-fucosidase	Lisosomial enzyme	870 nmol/ml	81%	70%
Glypican-3	heparin-sulfate proteoglycan (apoptosis regulator)	Positive	83.3%	96%
SCCA	Serine protease inhibitor	1.5 ng/ml	77.6%	84.4%

*EASL (European Association for Liver Study) data

explosion" of research involving biological tumor markers, also including hepato-biliary tumors. An ideal tumor-marker would be sensitive and specific enabling differentiation at an early stage of premalignant lesions from displastic ones (especially for HCC) and would also be easy to use, reproducible, cost-effective and minimally invasive(3). The aim of this work was to review the current knowledge of clinically applicable tumor markers in HCC, to explain the biological features, and to consider the possible applications in clinical practice considering the new molecules in this field.

3.BIOMARKERS

3.1.Primary liver tumours

The hepatocellular carcinoma is nowadays considered one of the commonest cancers worldwide with a rising incidence largely due to cirrhosis and the increase of HCV and HBV infections(4). HCC is responsible for about 700,000 deaths per year worldwide and even if prevalent in Southeast Asia and Africa, over the last decades has been on the increase in Europe and the USA(5). A recently published population study(6) showed that in the next two decades there will be a large increase of HCC incidence in the USA and Western Countries, mainly due to the diffusion of Hepatitis-related cirrhosis especially considering patients over 65 years old; a limit of this study is that it did not consider patients younger than 65 years, who make up the majority of HCC patients(7).

A modern diagnostic flow-chart of HCC normally includes many imaging methods, histological confirmation and serum markers(8). A large variety of Serum markers were proposed for diagnosis of HCC; the most common is the AFP (Alpha 1 fetoprotein). Other markers more recently isolated, studied and proposed for screening are: AFP variants (AFP L1, L2,L3 also named lens culinaris agglutinin-reactive AFP), Glypican-3, DCP, Alpha-1 – fucosidase, SCCA (squamous cell carcinoma agents)(Table 1), Hepatocyte growth factors (HGF) and Golgi protein 73. In addition TGF-B1 (Transforming growth factor-beta 1), VEGF (Table 2) and serum proteomics were recently considered in some studies as possible markers of HCC.

3.1.1.AFP (Alpha-1 fetoprotein)

First described by Abelev *et al.* in 1963(9) Alpha-1 fetoprotein is a glycoprotein usually fetal-specific, produced by the fetal liver under physiological conditions. This glycoprotein can also be produced by the fetal intestinal tract, the vitelline sac during the first 3 months of pregnancy, in fact, after birth its haematic level rapidly decreases because of the rapid regression of production. Its normal blood level in newborns at about 300 days after delivery is <10ng/ml(10). Also if highly expressed in patients affected by liver carcinoma, AFP is also highly produced by patients with nodular regeneration of the liver, and even more in patients affected by many tumors (lung, gastric cancers, embryonic carcinomas). Of course many patients can present a high level of AFP without being affected by a liver carcinoma. The sensitivity of AFP is very low (40-65%), with 40% false negatives(11) while the specificity is currently estimated at from 62 to 90%(12-14). The more appropriate cut-off of this marker has long been discussed in literature; a very low cut-off (>20 ng/ml) correlates badly with a very low sensitivity (54%)(11)(15); as known some conditions such as acute liver failure, mucoviscidosis, pregnancy and extended liver metastasis and, of course, acute and chronic hepatitis(10) can cause a rise of AFP up to 100 ng/ml(12). A better cut-off is considered 400-500 ng/ml for patients with cirrhosis(16) but at over 200 ng/ml its sensitivity dramatically decreases down to 22%(11). However, in a large study conducted by Farinati *et al.* only 18% of patients affected by HCC showed an AFP value >400 ng/ml and in this group of patients the overall survival was very poor(14). The cumulative predictive value of this marker is today estimated to be from 9 to 32%(17-19). Furthermore, AFP showed poor sensitivity for small lesions (25% sensitivity in lesions <3cm.), and unfortunately 80% of small HCC do not show an increase of AFP(18). For all these reasons Forner *et al.* argued that AFP should no longer be considered a useful marker of HCC especially for screening in which its usefulness had been demonstrated(20).

3.1.2. Lens-culinars agglutinin (LCA) reactive

These represent a relatively new family of markers mainly related to HCC. In fact, as reported the main problem of AFP is the lack of specificity because of its production even during HCV- and HBV-related cirrhosis, and this is a problem for HCC screening in cirrhotic patients in order to establish the appropriate cut-off over which we have to consider a patient at high-risk for HCC. Ninety-nine percent of patients with HCC are infected with HBV in China, this is an example of great correlation between these two diseases(21) and therefore clearly indicates the need to identify a biomarker for a differential serological diagnosis in this population of patients.

The LCA family is composed of 3 glycoproteins similar to AFP named AFP-L1, AFP-L2 and AFP-L3. AFP-L3 has LCA-binding activity, and has an additional α

Table 2. Other new biomarkers (genes, cytokines and receptors).

Biomarker	Biologic Features	Cut-off	Sensitivity	Specificity
GGT mRNA(Type B) (hystologic marker)	mRNA. Shift of predominance of Type B during HCC development	HCC Tissue expression (Type B)	-	-
hTERT	Reverse transcriptase of Human Telomerase (detectable in serum)	-	88.2%	70%
Alpha-fetoprotein mRNA (prognostic marker)	mRNA of Biomarker (prevision of recurrence)	Positive in serum after HCC resection	-	-
VEGF (prognostic marker)	Growth factor for stimulation of neovascularization	>245 pg/ml (in serum) (advanced HCC, bad prognosis)	n.a.	n.a.
TSGF (tumor specific growth factor)	Boold capillary amplification surrounding the tumor	62 U/ml	82%	-
IL-8 (serum marker)	Multifunctional chemochine involved in human neutrophil function (chemotaxis, enzyme release..), vascular endothelial proliferation and tumor migration	n.a.	n.a.	n.a.
GP 73 (Golgi Protein73)	Resident Golgi specific membrane Protein (up-regulated in HCC, cirrhosis and hepatitis9	10 relative units	69%	75%
HGF (Hepatocyte Growth factor) (prognostic marker)	Multifunctional factor involved in mitogenesis, cel motility, matrix invasion and carcinogenesis of epithelia	>0,6 ng/mL for cirrhotic patients	100%	100%
HSP 70 (Heat Shock Protein 70)	Hystological marker (up-regulated gene in HCC)	Unknown	Unknown	Unknown
CAP-2 (cyclase associated protein 2) (hystologic marker)	Dowstreaming ras and associated factor to adenylyl-cyclase.	n.a.	n.a.	n.a.
TGF-beta 1 (prognostic marker)	Multifunctional factor with a role regulation growth factor, angiogenesis, immunosuppression and carcinogenesisi	1,2 µg/L	89,5%	94%

1–6 fucose residue attached at the reducing terminus of *N*-acetyl-glucosamine. It appears to be produced only by cancer cells and is thought to be specifically related to HCC and not to cirrhosis or hepatitis, and is produced even at the early stages of tumor transformation, with a reported specificity of more than 95%(22). The L1 fraction of the total AFP is present in chronic hepatitis and liver cirrhosis, and constitutes a majority fraction of total AFP in non-malignant liver diseases. AFP-L2 is mostly derived from yolk sac tumors and could also be detected in maternal serum during pregnancy. AFP-L2 showed an intermediate affinity to LCA. These last two glycoproteins are not useful for detecting HCC. Furthermore, there is some evidence of a role of AFP-L3 as a marker of malignity of HCC; HCC-cells expressing this glycoprotein showed an earlier tendency to vascular invasion as well as a shorter doubling time compared to AFP-L3 negative ones(23)(24). The cut-off level of the AFP-L3 fraction is currently considered >10% as a predictive value for malignant transformation of a cirrhotic liver.

3.1.3. DCP (des-gamma-carboxy prothrombin) or PIVKA II (Protein induced by Vit.K absence II)

The Des-γ-carboxy prothrombin also known as prothrombin induced by Vitamin K absence II is a serum protein found under normal conditions only after a prolonged Vit.K deficiency, being stopped by the post-translational carboxylation of the prothrombin-precursor. This abnormal product of prothrombin precursor carboxylation also showed an autologous mitogen activity for HCC cell lines(25). This acquired defect was found to be present in malignant cells, and has therefore been proposed as a marker(26). The sensitivity of DCP is estimated from 48 to 62% and in some studies it provides no advantages compared with AFP(15)(27), however, it was proposed that the performance of these markers can be additive, improving the sensitivity for screening of HCC(28). The most commonly used cut-off value for DCP is 40 mAU/ml in serum; however, some Authors reported an increase of sensitivity and specificity for differentiating HCC from non-malignant chronic liver diseases with a higher cut-off (125 mAU/ml) of respectively 89% and 86.7%(29).

It was suggested that the surveillance of HCC should be managed by the combination of more than one marker in order to improve the efficacy (sensitivity and sensitivity); so that the contemporary determination of DCP (with low cut-off 40 mAU/ml), AFP (125 ng/ml) and AFP-L3 (cut-off value up to 10%) can reach 82-3% accuracy(30). In countries with a high prevalence of HCC such as Japan (the Japanese national health insurance covers patient's cost for determination of AFP AFP-L3 and DCP(31)) this method increases the sensitivity maintaining a high specificity for tumors < 2 cm. Of course marker determination alone is not enough for HCC screening and it must be associated with periodical US-scans, and periodically (every 6-12 months) CT and MRI for cirrhotic or obese patients difficult to evaluate only with US-scan(32)(33).

3.1.4. Alpha-1-fucosidase.(AFU)

AFU is a lisosomial enzyme and its biological role in cells is to hydrolyze fucoglyco-coniugates(34), its activity was found to be increased in patients affected by HCC, cirrhosis and chronic hepatitis(35). As for other biomarkers, the main problem is the cut-off, conditioning the sensitivity and specificity especially in the early detection of HCC.

It was established that using a cut-off of 870 nmol/mL the sensitivity is about 81% and specificity around 70%(36), despite the fact that in one prospective study a sensitivity of 82% and a specificity of 100%(37) was reported, this remains to be confirmed. Furthermore, the AFU activity is elevated in other malignancies such as

ovarian and colorectal cancer; and it was found to be elevated also in other benign diseases (hypothyroidism, diabetes and pancreatitis)(38)(39). In addition AFU is useful in the detection of cirrhotic patients becoming highly positive in 85% of patients' sera at least 6 months before developing the first ultrasonographic signs of cirrhosis(35), and this seems to be a promising clinical application of this biomarker.

3.1.5. Glypican-3 (GPC 3)

A new biomarker is GPC-3 that is a heparin-sulfate proteoglycan normally present as a cell-suface protein, as a member of the glypican family(40)(41), it is an oncofetal protein inactive in the adult liver, but re-activated in HCC tissues. Its m-RNA is normally not detectable in human tissues except for fetal liver and placenta, but it was discovered to be present in HCC. More recently GPC-3 has been believed to be expressed also in Yolk-sac tumors, seminomas (having a role in testicular germ cell tumor differentiation), lung cell tumors, clear cell carcinomas of the ovary(42) and melanomas(43). Its function is also related to the regulation of growth-factor effects(44)(45), particularly as an inhibitor of apoptosis(46) being involved in the pathogenesis of Simpson-Golabi-Behmel syndrome due to an X-linked disorder inducing a liver and other organ overgrowth(46). The key point is that this glycoprotein is over-expressed on the cell-surface of small HCC but not in cirrhotic or regenerative nodules(31).

The serum levels of GPC-3 are significantly higher than healthy adults in about 53% of HCC patients, and in 33% of HCC patients negative for AFP and DCP. Therefore if the combination of sera level determination of both biomarkers (GPC-3 and AFP) can increase the sensitivity to about 72% with a lowering of the specificity(47), it is interesting to note that the determinations on cytological materials after FNA-Biopsy showed a sensitivity of 83.3 % and a specificity of 96%, enabling a very clinically important differentiation between a small regenerative nodule, benign disease and a small hepatocarcinoma nodule(48)(49). Furthermore, in a recent study conducted on 107 patients affected by HCC, it was demonstrated that GPC-3 + patients had a statistically significative poor prognosis compared with the GPC-3- ones, showing a possible role of GPC-3 as a prognostic factor, especially indicating a risk of an early intrahepatic recurrence(50). Among the new biomarkers GPC-3 seems to be one of the more promising, even if there are few studies on the real clinical impact of such a biomarker.

3.1.6. SCCA (squamous cell carcinoma agents)

This is a relatively little known and studied serine protease inhibitor, physiologically expressed in skin and squamous epithelial cells, but also in cervix, lung and head and neck malignancies(51). Abnormal over-expression of SCCA 1 and 2 variant antigens is found in HCC specimens while no expression is normally detected in healthy liver tissue(52)(53). To date there have been few studies considering SCCA as a tumor marker, the main advantage seems to be in the detection of early phases of HCC, when even AFP is negative but tumor growth has just started(54). Using 1.5 ng/ml as cut-off the sensitivity and specificity of this marker are respectively 77.6% and 84.4%(54). The simultaneous determination of AFP and SCCA has been proposed to improve the specificity and to also increase the sensitivity especially in the early phases of HCC growth(55). Furthermore, the underlying liver disease seems to be important in choosing the most appropriate biomarker because, for example, Beale reported that SCCA 1 is not elevated in patients affected by hepatocarcinoma with alcoholic or non alcoholic fatty liver disease, suggesting a role of this biomarker in HCV-related HCC(56).

An increase of this biomarker in patients' sera was observed during progression from pre-neoplastic lesions (dysplastic lesions) to malignant tumors, but with the enlargement of the lesion an over-expression in the peri-tumoral tissue and a decrease in serum levels were observed, in agreement with the observation that SCCA is not a secreted molecule; therefore it should be useful in the early detection of malignant transformation, showing limits in clinical applications because of its mainly tissue expression in late phases of tumor growth, with a still unclear correlation between serum- and tissue-levels(57).

3.1.7. Others

In the last 10 years other molecules have been variously correlated with HCC transformation or growth, and therefore studied as possible clinical tumor markers for HCC screening, but to date a very small number of studies is available for these markers so that we are induced just to cite them only to give an idea of possible future developments in this field, but we feel that these markers are still far from clinical applications. To date most of them have shown a role as prognostic factors instead of biological markers, being spies of malignant biological features of HCC. They are summarized in Table 2.

4. CONCLUSIONS

The screening of HCC is nowadays gaining more importance even in Western Countries because of the raising up of HCC in the last two decades. Despite the big number of published data, till today we are still far from the identification of the ideal biomarker for early detection of HCC especially on cirrhotic patients. For an early diagnosis of HCC the most satisfactory strategy based on biomarkers seems to be, till today, the simultaneous detection of AFP, AFP-L3 and DCP together with periodical US screening as in Japan codified, A further diagnostic imaging for doubtful cases integrated by FNAB or biopsy was also stated from EASL (European association for the study of the liver), according also to AASLD (American Association for Study of Liver Disease) 2005 guidelines. From the available data GP-3, SCCA and Alpha-1-fucosidase seem to be very promising molecules that will find clinical applications in the future. For the other biomarkers (see Table 2) more studies are needed in order to evaluate their real clinical role and usefulness.

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Abbreviations: HCC: Hepatocellular Carcinoma, AFP: Alpha-fetoprotein, AFP-L3 :Alpha-fetoprotein, Isoform 3 of . Lens-culinars agglutinin), LCA : Lens-culinars agglutinin, DCP :des-gamma-carboxy prothrombin, , PIVKA II: Protein induced by Vit.K absence II, HCV: Hepatitis C Virus, US : Ultrasound, HBV: Hepatitis B Virus, GPC 3 : Glypican-3, SCCA: Squamous Cell Carcinoma Agents, FNA: Fine Needle Aspiration, FNAB: Fine Needle Aspiration Biopsy, VEGF: Vascular EndothelialGrowth Factor, hTERT: Reverse Transcriptase of Human Telomerase, TSGF: Tumor specific growth factor , GP 73: Golgi Protein73, HGF: (Hepatocyte Growth factor, HSP 70: Heat Shock Protein 70, CAP-2 (cyclase associated protein 2)
TGF-beta 1: Tumor Growth Factor beta 1.

Key Words: Biomarkers, HCC, Hepatocellular Carcinoma, Bioumoral Screening, Review

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