metastasis, and recurrence has attracted great deal of research interest resulting in discovery and utilization of several novel markers in this disease. In this paper we try to give an overview of available data on this burgeoning area of research.

2. Biomarkers for Liver Cancer

2.1. Oncofetal and Glycoprotein Antigens

2.1.1. Alpha-Fetoprotein (AFP). The first serologic assay for detection and clinical followup of patients with hepatocellular carcinoma was alpha-fetoprotein (AFP) which has been the standard tumor biomarker for HCC for many years. It is a glycoprotein produced by the fetal liver and yolk sac during pregnancy. Serum AFP levels are often elevated in HCC, but this is not always the case. AFP levels may be elevated initially in the early stages of HCC and then drop or even normalize before rising again as disease progression occurs [5]. Additionally, AFP elevation has also been recognized in the presence of acute and chronic viral hepatitis as well as in patients with cirrhosis caused by hepatitis C. Given the multiple indications that present with elevated AFP levels, it is necessary to evaluate the significance of serum concentrations. In general, consistently elevated serum AFP levels greater than 500 ng/mL are indicative of HCC. Lower serum concentrations which are only transient in nature are more often present in benign liver disease [6]. If a patient has known risk factors for HCC, such as the presence of cirrhosis, increasing levels of AFP have been shown to correlate with the development of HCC [6]. Unfortunately, AFP serum concentrations do not correlate well with the prognostic values of HCC such as tumor size, stage, or disease progression, and ethnic variability may also exist. Furthermore, in some cases of HCC, AFP elevations are not apparent at all [7]. Total AFP can be divided into three different glycoforms, AFP-L1, AFP-L2, and AFP-L3-based on their binding capability to lectin *Lens culinaris* agglutinin (LCA). High percentage of AFP-L3 has been shown to be associated with poor differentiation and biologically malignant characteristics, worse liver function, and larger tumor mass [8].

2.1.2. Glypican-3. Glypican-3 (GPC3), a membrane-anchored heparin sulfate proteoglycan, has been demonstrated to interact with growth factors and modulate their activities. It binds to the cell membrane through the glycosylphosphatidylinositol anchors. GPC3 mRNA was upregulated significantly in tumor tissues of HCC compared to paraneoplastic liver tissue, liver tissues of healthy adults, and liver tissues of patients with nonmalignant hepatopathy. The expression of GPC3 (at both mRNA and protein levels) in the serum of HCC patients was significantly higher than that in the serum of healthy adults or patients with nonmalignant disease. It can be detected in 40–53% of HCC patients and 33% of HCC patients seronegative for both AFP and Des-gamma-carboxyprothrombin (DCP) [9, 10]. It has been shown that soluble GPC3 (sGPC3), the NH_2-terminal portion of GPC3, is superior to AFP in the sensitivity of detecting well or moderately differentiated HCC, and the simultaneous determination of both markers improves overall sensitivity from 50% to 72%. Recently, a study compared the survival rate between the GPC3-positive and GPC3-negative HCC patients. GPC3 positivity correlated with poor prognosis and identified as an independent prognostic factor for the overall survival on multivariate analysis [11].

Table 1: Diagnostic values of HCC serum markers [12–14].

Type of test	Sensitivity (%)	Specificity (%)
AFP-L3	61.6	92.0
DCP	72.7	90.0
AFP	67.7	71.0
AFP-L3 + DCP	84.8	97.8
AFP-L3 + AFP	73.7	86.6
DCP + AFP	84.8	90.2
AFP-L3 + DCP + AFP	85.9	59.0

2.2. Enzymes and Isoenzymes

2.2.1. Des-Gamma-Carboxy (Abnormal) Prothrombin (DCP). DCP is produced by the malignant hepatocyte and appears to result from an acquired posttranslational defect in the vitamin-K-dependent carboxylase system. DCP production is independent of vitamin K deficiency, although pharmacological doses of vitamin K can transiently suppress DCP production in some tumors. DCP levels greater than 0.1 AU/mL (100 ng/mL) on ELISA are highly suggestive of HCC or tumor recurrence. Normalization of DCP levels correlates well with successful tumor resection and appears to be an excellent marker of tumor activity. It is thought that the combination of AFP and DCP assays will increase the sensitivity of testing. The correlation between tumor size and DCP levels is not yet clearly defined. It appears that there is a correlation in DCP levels and large tumors; however, the same is not the case in small tumors (<3 cm) [15]. A cross-sectional case control study involving 207 patients determined that DCP is more sensitive and specific than AFP for differentiating HCC from nonmalignant liver disease. In this study there were 4 groups studied: normal healthy subjects; patients with noncirrhotic chronic hepatitis, patients with compensated cirrhosis, and patients with histologically proven HCC. Both DCP and AFP levels increased among the groups as disease severity increased (from normal to HCC), but DCP values had less overlap among the groups than AFP. Study results concluded that a DCP value of 125 mAU/mL yielded the best sensitivity and specificity for differentiating patients with HCC from those with cirrhosis and chronic hepatitis [16]. Sensitivity and specifity of total AFP, AFP glycoforms, DCP, and combinations of both markers have been summarized in Table 1.

2.2.2. Gamma-Glutamyl Transferase. Serum gamma-glutamyl transferase (GGT) in healthy adults is mainly secreted by hepatic Kupffer cell and endothelial cell of bile duct,