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Case Series

Early Detection of Hepatocellular Carcinoma Recurrence Using the Highly Sensitive Fucosylated Fraction of Alpha-Fetoprotein

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Abstract
Alpha-fetoprotein (AFP)-L3 was originally reported as a hepatocellular carcinoma (HCC)-specific tumor marker, and recent accumulation of evidence has revealed that AFP-L3 frequency predicts the biological malignancy potential of HCC. However, AFP-L3 elevation from undetectable levels after curative treatment could not be discussed due to the difficulties of calculating AFP-L3 concentrations when serum AFP levels were low. Here, as a novel method, we used highly sensitive AFP-L3 frequency to predict HCC recurrence after curative treatment. Our cases illustrate that recognizing elevation of AFP-L3 from undetectable levels led to the early detection of recurrent HCC due to more careful surveillance.

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Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related deaths worldwide and has a high recurrence rate, even after curative treatment [1]. Alpha-fetoprotein (AFP), the fucosylated fraction of AFP (AFP-L3), and des-gamma-carboxy prothrombin (DCP) have been widely used as tumor markers for HCC. While AFP-L3 was originally reported as an HCC-specific tumor marker, recent accumulation of evidence has revealed that AFP-L3 frequency predicts the biological malignancy potential of HCC, such as portal vein invasion, lower tumor classification, and advanced tumor stage, regardless of whether there is a small tumor size and/or lower serum AFP concentration [2]. Tateishi et al. [3] reported that, based on the results of a multivariate analysis, an AFP concentration >100 ng/mL and an AFP-L3 concentration >15%, both pre- and post-percutaneous ablation, were significant predictors of HCC recurrence. Saito et al. [4] reported that the preoperative AFP-L3 value in patients with early recurrence (within 1 year after hepatectomy) was significantly higher than that in those without recurrence. Tamura et al. [5] reported that, due to the high recurrence and poor prognosis of patients with HCC with an AFP-L3 concentration >15%, patients with this AFP-L3 concentration should be treated with careful consideration. Furthermore, Matsuda et al. [6] reported that AFP-L3 is a useful prognostic biomarker for survival after repeated hepatic resection for HCC. They reported that the 1-, 3-, and 5-year survival rates after the second hepatic resection of 27 patients with low AFP-L3 concentrations (≤15%) were 100, 100, and 91.7%, respectively, whereas the corresponding survival rates for 8 patients with HCC with high AFP-L3 concentrations (>15%) were 100, 47.6, and 23.8%, respectively [6]. Recently, a highly sensitive immunoassay using on-chip electrokinetic reaction and separation by affinity electrophoresis (micro-total analysis system; μTAS) for AFP-L3 was developed, and AFP-L3 frequency can now be measured accurately at very low AFP concentration levels [7]. To date, although AFP-L3 levels are reported to predict the malignant potential of HCC and although sustained elevation of AFP-L3 after curative treatment indicates HCC recurrence, AFP-L3 elevation from undetectable levels after curative treatment could not be discussed due to the difficulties of calculating AFP-L3 concentrations when serum AFP levels were low. Here, as a novel method, we used highly sensitive AFP-L3 frequency to predict HCC recurrence after curative treatment. In our cases, elevation of AFP-L3 from undetectable levels after curative treatment predicted HCC recurrence from very low serum AFP levels.

Case 1

A 49-year-old man with chronic hepatitis B infection treated with lamivudine and adefovir underwent a right anterior segment hepatectomy followed by two rounds of radiofrequency ablation therapy in segments 4 and 7 to treat HCCs that were 7 mm and 13 mm in diameter, respectively. Two years after the last radiofrequency ablation therapy, he developed lower back pain. Laboratory findings showed an elevated serum AFP level and AFP-L3 frequency (19 ng/mL and 39.3%, respectively). Magnetic resonance imaging (MRI) revealed tumor metastasis in the 12th thoracic vertebra, which was resected and diagnosed as an HCC metastasis. Four months after that operation, his serum AFP level and AFP-L3 frequency returned to the normal range (1 ng/mL and <0.5%, respectively). However, 14 months after the operation, laboratory findings showed an elevated AFP-L3 frequency (26.3%), while serum AFP levels remained at 3 ng/mL. AFP-L3 frequency tended to increase with time,

while the AFP level remained within the normal range for several months. Although neither computed tomography (CT) nor MRI showed any abnormalities, positron emission tomography (PET)-CT did reveal hot spots at the first lumbar vertebra (Fig. 1).

Case 2

An 80-year-old man with cryptogenic chronic hepatitis and chronic kidney disease requiring hemodialysis developed an HCC in the right lobe that was 78 mm in diameter and underwent an extended right hepatectomy. His pre-operative serum AFP level, AFP-L3 frequency, and DCP level were 16,473 ng/mL, 80.5%, and 2,257 mAU/mL, respectively. Five months after the surgery, his serum AFP levels and AFP-L3 frequency returned to within the normal range (1 ng/mL and <0.5%, respectively). CT performed at the same time demonstrated no recurrence. However due to the high AFP-L3 frequency before the operation, very careful recurrence surveillance was performed. Seven months post-treatment, the AFP-L3 frequency increased to 28.6%, while the serum AFP (only 2 ng/mL) and DCP levels remained within the normal range. Eight months after surgery, however, the AFP-L3 frequency reached 47.8%, while serum AFP and DCP levels remained within the normal range, at 7 ng/mL and 26.1 mAU/mL, respectively. We suspected HCC recurrence based on the elevation of AFP-L3 frequency, which resulted in detection of HCC recurrence by CT at an earlier date than would have occurred without this test (Fig. 2).

Discussion

Early detection of HCC recurrence after curative treatment is a very critical problem that influences patient prognosis. Thus, effective surveillance to detect HCC recurrence is urgently needed. HCC is a quite heterogeneous and diverse tumor. Although very heterogeneous genetic abnormalities of HCCs have recently been elucidated [8] and some researchers have tried to predict their biological malignancy behavior using hepatic progenitor markers such as cytokeratin 19 [9], epithelial cell adhesion molecules [10], and neural cell adhesion molecules [11, 12], clinically conventional serum tumor markers are the most frequently used markers at this point. While serum tumor markers, AFP, AFP-L3, and DCP have been widely used and imaging modalities have become more advanced for detecting HCC, several difficulties in detecting early recurrence still exist. Regarding AFP and DCP, insufficient sensitivity and specificity have been problematic, and AFP-L3 could not be calculated when the serum AFP levels were low. Recently, a highly sensitive AFP-L3 immunoassay was developed that overcomes the previous difficulty in accurately measuring AFP-L3 at very low AFP concentrations [7]. AFP-L3 levels reportedly predict the biological malignancy potential of HCC, and sustained elevation of AFP-L3 after curative treatment indicates HCC recurrence. However, no report has clearly indicated that elevation of AFP-L3 from undetectable levels after curative treatment can predict HCC recurrence a few months earlier than AFP and DCP assessment can. Given that HCCs that elevate the serum AFP-L3 level are known to have malignant potential and result in poor patient prognosis, it is reasonable to monitor AFP-L3 levels to detect early HCC recurrence. Generally, it is rare for AFP-L3 to become negative after treatment. Kobayashi et al. [13] reported the relationship between changes in the serum AFP-L3 level measured by the highly sensitive immunoassay 30–120 days after curative treatment and HCC recurrence. They concluded that 29 of 37 patients (78.4%) with preoperative AFP

elevation (>20 ng/mL) showed a decrease in the AFP level to <20 ng/mL, although 16 of 42 patients (38.1%) with preoperative AFP-L3 elevation (>5%) showed a decrease in the AFP-L3 level to <5% [13]. In our cases after curative surgery, AFP-L3 decreased to within undetectable levels even using the highly sensitive AFP-L3 immunoassay. Afterward, using the highly sensitive AFP-L3 immunoassay, we could detect elevation of AFP-L3 frequency from very low serum AFP levels: 3 ng/mL (case 1) and 2 ng/mL (case 2). Recognizing the elevation of AFP-L3 led to the early detection of recurrent HCC due to more careful surveillance using imaging tests, such as CT and MRI, in these two cases. In addition, in case 1, PET-CT was a useful tool for detecting extrahepatic metastasis. Based on our experience with these cases, we recommend that, once elevated AFP-L3 frequency is detected from previously undetectable levels, strict surveillance of HCC recurrence should be performed, which we suspect will result in increasing the possibility that the next treatment will be curative. In conclusion, measuring AFP-L3 not only allows prediction of the biological malignancy potential of HCC before treatment, but it also allows for the detection of HCC recurrence after curative treatment earlier than the measurement of AFP and DCP can.

Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors declare no conflict of interest.

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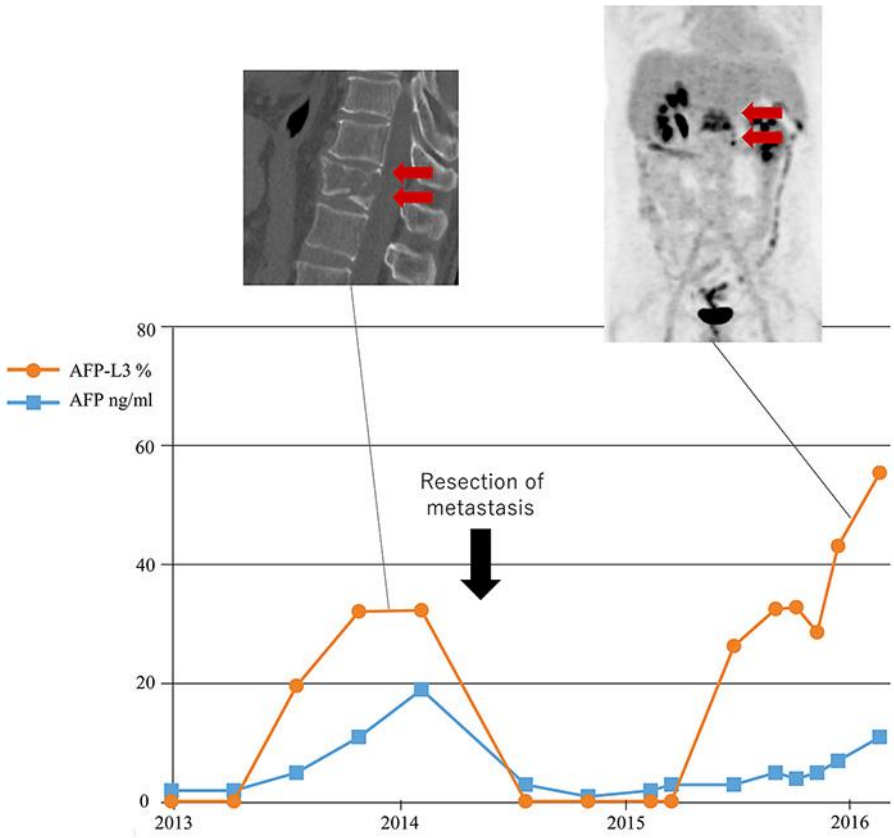

Fig. 1. Clinical course of case 1. After the operation of bone metastasis, his serum AFP level and AFP-L3 frequency returned to the normal range. Fourteen months after the operation, only AFP-L3 started to elevate and then reappearance of bone metastasis was confirmed by PET-CT.

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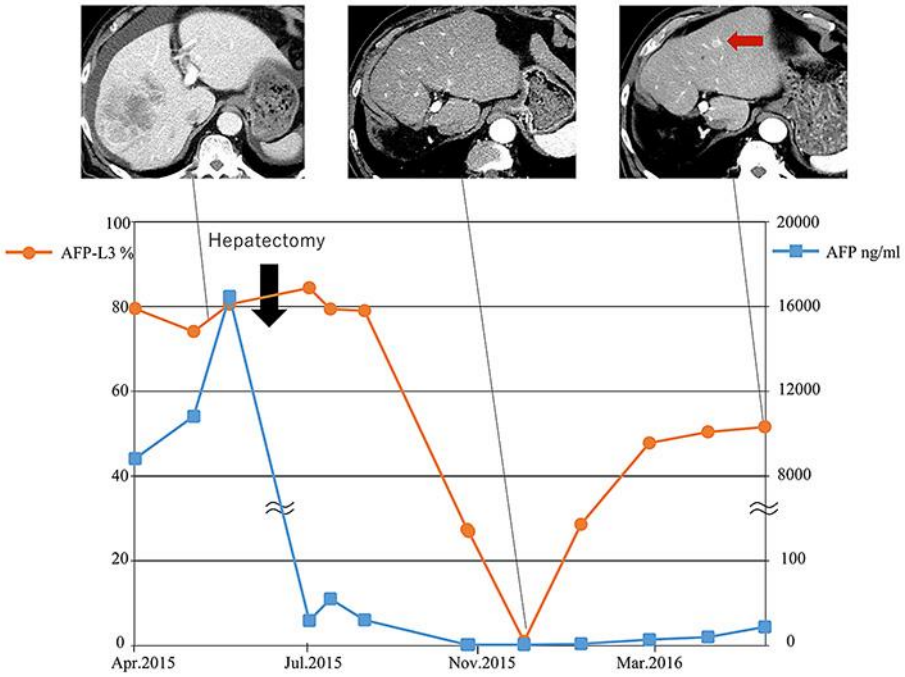

Fig. 2. Clinical course of case 2. After the operation of huge HCC, his serum AFP level and AFP-L3 frequency returned to the normal range. Seven months post-treatment, the AFP-L3 frequency increased again, while the serum AFP and DCP levels remained within the normal range. Soon after this AFP-L3 elevation, intrahepatic metastasis was confirmed by CT.