TEMs but Not DKK1 could Serve as Complementary Biomarkers for AFP in Diagnosing AFP-negative Hepatocellular Carcinoma

yueguo wang

Abstract

Background & Aims Hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) is prevalent worldwide. Despite its limitations, serum alpha-fetoprotein (AFP) remains the most widely-used biomarker for the diagnosis of HCC. This study aimed to assess whether measurement of peripheral plasma Dickkopf-1 (DKK1) and Tie2-expressing monocytes (TEMs) could overcome the limitations of AFP and improve the diagnostic accuracy of HCC.

Methods Plasma DKK1 level and the percentage of TEMs in peripheral CD14+CD16+ monocytes from HCC patients (*n*=82), HBV-related liver cirrhosis (LC) patients (*n*=29), chronic hepatitis B (CHB) infected patients (*n*=28) and healthy volunteers (*n*=31) were analyzed by ELISA and flow cytometry. Receiver operating characteristic (ROC) curves were used to analyze a single biomarker, or a combination of two or three biomarkers.

Results The percentage of TEMs in peripheral CD14+CD16+ monocytes and plasma level of DKK1 in HCC group were significantly higher than those in LC, CHB and healthy control groups (all *P*-values <0.05). The percentage of TEMs alone was also significantly higher in AFP-negative HCC group than that in LC, CHB and healthy control groups (all *P*-values <0.05). Plasma DKK1 level alone could not distinguish between AFP-negative HCC and LC patients. ROC curves showed that the optimal diagnostic cutoff value was 550.93 ng/L for DKK1 and 4.95% for TEMs. There was no significant difference in AUC of DKK1, TEMs and AFP in HCC diagnosis between the four groups (all *P*>0.05). A combination of DKK1, TEMs and AFP measurements increased the AUC for HCC diagnosis as compared with either marker alone (0.833; 95%CI 0.768-0.886). The AUC for TEMs was 0.692 (95% CI 0.564-0.819) in differentiating AFP-negative HCC from LC, with a sensitivity of 80.0% and a specificity of 65.52 %.

Conclusions TEMs and DKK1 may prove to be potential complementary biomarkers for AFP in the diagnosis of HCC. TEMs rather than DKK1 could serve as a complementary biomarker for AFP in the differential diagnosis of AFP-negative HCC versus LC patients.

Citation: yueguo wang TEMs but Not DKK1 could Serve as Complementary Biomarkers for AFP in Diagnosing AFP-negative Hepatocellular Carcinoma. **protocols.io**
dx.doi.org/10.17504/protocols.io.irhcd36
Published: 07 Jul 2017

Materials

Protocol for Analysis of Tie2-expressing Monocytes by Contributed by users

Protocol

1. Sample preparation – Collect 3 ml of whole blood of patients with empty stomach using heparin as an anticoagulant before treatment. Do the experiment as soon as possible in 2 hours.

Step 1.

2. Take two special test tubes for flow cytometry. Mark the tubes with testing number +B and testing number +T respectively with a marker pen.

Step 2.

3. Mix each specimen thoroughly by slow inversion of the sample tube 10 times. Add 100 µl of whole blood to the testing number +B and testing number +T tubes respectively.

Step 3.

4. Add 20 µl of FcR blocking reagent to each testing tube. Mix tubes by middle speed vortexing for 5 - 10 seconds. Incubate for 20 minutes at room temperature and protect from light.

Step 4.

5. Add 10µl of CD14- FITC mouse anti-human antibody to each testing tube.

Step 5.

6. Add 10µl of CD16-PE-Cy5 mouse anti-human antibody to each testing tube.

Step 6.

7. Add 1 µl of TIE2-PE mouse anti-human antibody to each testing number +T tube.

Step 7.

8. Add 1 µl of IgG1-k-PE mouse anti-human antibody to each testing number +B tube.

Step 8.

9. Mix each testing tube thoroughly by middle speed vortexing for 5 - 10 seconds.

Step 9.

10. Incubate for 20 minutes at room temperature in the dark .

Step 10.

11. Add 600 µl of hemolysis reagent A (0.12% methanoic acid) to each testing tube. Mix each testing tube thoroughly by middle speed vortexing for 5 - 10 seconds. Add 270 µl of hemolysis reagent B (Na2CO3 6.0 g/L□NaCl 14.5 g/L□Na2SO4 31.3 g/L) to each testing tube. Mix each testing tube thoroughly by middle speed vortexing for 5 - 10 seconds.

Step 11.

12. Add 1.5 ml of PBS to each testing tube. Blow gently and mix evenly.

Step 12.

13. Centrifuge the tubes at 1500 rpm for 5 minutes.

Step 13.

14. Remove each tube from the centrifuge and inspect for the presence of a well-formed white film at the bottom of the tube and pour off the supernatant .

Step 14.

15. Add 2.0 ml of PBS to each testing tube. Blow gently and mix evenly.

Step 15.

16. Centrifuge the tubes at 1500 rpm for 5 minutes.

Step 16.

17. Pour off the supernatant. Add 0.5 ml of PBS and blow gently and mix evenly. Tie2-expressing monocytes (TEMs) are detected immediately by Flow cytometry. Generally, at least 10 000 CD14+

monocytes per sample are counted and analyzed.

Step 17.

18. The percentage of TEMs in CD14+CD16+ monocytes is evaluated by TIE2-PE staining minus that evaluated by IgG1-k-PE isotype control.

Step 18.