Optical Biopsy of Liver Fibrosis using Multiphoton Microscopy

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Abstract- In this work, we apply multiphoton microscopy in diagnosing toxin (CCl₄) induced liver fibrosis in mice. We found that multiphoton induced spectroscopic signatures may be used to identify stellate cell proliferation and necrotic sites. Our study suggests that multiphoton imaging may be developed into an effective diagnosis tool for liver fibrosis in vivo.

Introduction

Liver diseases are serious problems to human health. Conventional histological procedures limit the ability to diagnose liver tissues in living specimens, and the development of intravital imaging techniques is invaluable in following liver physiology in vivo. The purpose of this study is to test the capabilities of multiphoton imaging in the diagnosis of liver fibrosis in vivo, thus, lead to early detection and treatment.

Materials and Methods

We used the 780 nm output of a femtosecond, titanium-sapphire laser as the excitation source. The laser is scanned by an x-y scanning system. After being processed by the optical instruments and the microscope, images are collected by the PMT and recorded by the computer.

Liver fibrosis was induced from male BALB/c mice injected with CCl₄. Starting at 6 weeks of ages, the mice were given intra-peritoneal injections of 1:9 volumetric ratios of CCl₄ and olive oil. Twice weekly, the dosage of 1 μ l CCl₄ per gram of mouse body mass was applied. The mice were sacrificed 24 hours after the final injection and the left medial lobe of the liver was removed to be imaged from the surface using the multiphoton microscope described above.

Results and Conclusions

Multiphoton images of normal and mice livers after 1, 2, and 3 weeks of CCl₄ induction are shown in Figs. 1-4(next page). Distribution of hepatocytes (elliptically enclosed regions) and stellate cells in normal mouse liver and those treated with 1, 2, and 3 weeks of CCl₄ (yellow arrow: a stellate cell) are identifiable.

Left: magnified multiphoton image. Center: large area multiphoton scan (1.27 mm by 1.21 mm). Right: histological images (hepatocyte nuclei: purple; stellate cells: red). The scale bar is 100 μ m.

We have shown that multiphoton microscopy is capable of identifying liver damage in mice following the treatment of CCl₄. Cell necrosis and the proliferation of stellate cell at the damaged sites can be identified under multiphoton imaging. Our results suggest that multiphoton imaging may be developed into effective diagnostic tool for liver fibrosis in vivo.

References

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0 1

Fig.1 Normal liver

0 1

Fig.2 1 week induction

0 1

Fig.3 2 weeks induction

0 1

Fig.4 3 weeks induction