Evaluating Rat Non-alcoholic Fatty Liver Disease (NAFLD) by Raman Spectroscopy and Machine Learning Method.

Yoshinori Harada\textsuperscript{a}, Khalifa Mohammad Helal\textsuperscript{b}, Harsono Cahyadi\textsuperscript{a}, J. Nicholas Taylor\textsuperscript{b}, Koji Tabata\textsuperscript{b}, Yasuaki Kumamoto\textsuperscript{a}, Tetsuro Takamatsu\textsuperscript{a}, Hideo Tanaka\textsuperscript{a}, Atsuyoshi Nakamura\textsuperscript{a}, Katsumasa Fujita\textsuperscript{d}, Tamiki Komatsuzaki\textsuperscript{b}

\textsuperscript{a}Department of Pathology and Cell Regulation, Kyoto Prefectural University of Medicine, Kyoto, Japan; \textsuperscript{b}Research Institute for Electronic Science, Hokkaido University, Sapporo, Japan.; \textsuperscript{c}Graduate School of Information Science and Technology, Hokkaido University, Sapporo, Japan; \textsuperscript{d}Department of Applied Physics, Osaka University, Osaka, Japan.

NAFLD is classified into two disease categories, nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). NAFL basically has benign prognosis. In contrast, NASH generally develops liver fibrosis and occasionally occurs with liver cirrhosis and hepatocellular carcinoma. However, some cases histologically diagnosed as NAFL by using liver biopsied specimens develop to NASH. It is difficult to predict future onset of NASH from histopathological findings. Raman spectroscopy that can acquire molecular information has the potential to identify nascent state of NASH. Here, we sought to analyze Raman spectral features of livers of rat NAFLD models by using a machine learning method and to investigate whether it is possible to detect a nascent state of NASH.

METHODS: Eight-week-old rats were divided into 3 groups fed with standard diet (SD), high-fat diet (HFD), or high-fat high-cholesterol diet (HFHC) for 2, 4, 8 and 16 weeks, respectively. After excision of liver tissues, Raman spectroscopic analysis combined with a machine learning method, random forest, and histopathological analyses were carried out.

RESULTS: Liver histology showed normal findings in the HFD group and NAFL in the HFHC group at 2 weeks of feeding, and NAFL in HFD group and NASH in the HFHC group at 16 weeks of feeding. Raman peaks and chemical substances important for classification of disease type were extracted by random forest and mass spectroscopy, and rats under nascent state of NASH could be identified.

CONCLUSIONS: Precise evaluation of liver tissues of NAFLD was achieved by using Raman spectroscopy and machine learning method.