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Background: Cholangiocarcinoma (CCA) is a major health problem in Thailand especially in the northeast. Advanced imaging technology and tumour markers are used in the diagnosis but not highly specific for CCA diagnosis. Fourier transform infrared (FTIR) microspectroscopy is the upcoming powerful technique to diagnose several diseases. This study aims to characterize the infrared spectral pattern of CCA cells from normal cholangiocytes in both of cell lines and human tissues which can provide the specific spectral signature for CCA diagnosis. **Methods:** Cell lines including immortal cholangiocyte cell line (MMNK-1) and CCA cell lines (KKU-214 and KKU-139) were cultured onto sterile transfection slides. For human tissues, frozen CCA and normal adjacent tissues were cryosectioned at 4 µm thickness and placed onto transfection slides. The parallel tissue sections were stained with H&E for area identification. All samples were prepared for FTIR microspectroscopy measurement. Samples were detected with synchrotron source at Australian Synchrotron using 10 µm × 10 µm aperture size with 4 cm⁻¹ spectral resolution. The spectral analysis was performed with OPUS software and principal component analysis (PCA) by The Unscrambler[®] X software at C-H stretching and fingerprint region.

Results: The spectral region at C-H stretching can classify the CCA cells in both of cell lines and tissues better than a fingerprint region. The major infrared spectral signature of CCA showed peaks at 2,921 cm⁻¹ of CH2 asymmetric stretching and 2,852 cm⁻¹ of CH2 symmetric stretching modes from carbohydrates and lipids. On the other hand, the cholangiocyte showed increased intensity at 2,875 cm⁻¹ of CH3 symmetric stretching.

Conclusions: This FTIR technique shows potential for characterization of the non-cancerous and cancerous CCA cells. Therefore, the useful of infrared spectral pattern may be used as a marker for CCA diagnosis. However, more tissues samples size should be used for further investigation. **Keywords:** Cholangiocarcinoma (CCA); infrared spectrum;

Fourier transform infrared (FTIR); diagnosis; multivariate analysis

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