2014 年度修士論文要旨

# Label-free *in situ* monitoring for effects of anticancer drugs in cancer cell by using Raman spectroscopy

関西学院大学大学院理工学研究科
国際修士プログラム　生命科学専攻　佐藤研究室　Sameerah Alzahrani

Raman spectroscopy is one of the most promising techniques for studying cancer and the effects of its chemical treatments. Raman spectroscopy is based on the inelastic scattering of photons by molecule, which provide information of molecular composition in cells or organelles without sample preparation. The technique monitors and assesses cellular condition rapidly and nondestructively. It has relatively high spatial and temporal (seconds to minutes) resolutions. In this study, we investigated how Raman spectroscopy is sensitive and reliable to detect responses caused by the administration of caffeine in living cancer cells and normal cells, which is one of the most popular candidates of natural materials for prevention of cancer. Transmission electron microscopy (TEM) was applied also to detect the morphological changes due to the caffeine and comparing anticancer drug. Consequently, the present study successfully demonstrated that Raman spectroscopy is a powerful technique for studying the effect of anticancer drugs in live cells. Furthermore, these techniques may help reduce the need for animal experiments. Human pancreatic cells (BxPC-3: cancer cell line; IC3D3 normal cell line) were used. These cells were treated with various concentrations of caffeine (10, 50 and 100 μg/ml). 5-FU was used for comparison study with various concentrations (1, 10 and 100 μg/ml). Raman measurement were carried out at 24 and 48 h after the treatments. The Raman spectra of the control and treatment groups were measured under the same incubation conditions in a confocal Raman microscope (Nanofinder® 30, Tokyo Instruments), which has x60 objective lens, 600/750nm grating and an electronically tuned Ti:Sapphire laser at 785 nm wavelength. The laser power was 30 mW at the sampling point. The Raman spectra were analyzed with principal component analysis (PCA). TEM was used to observe changes in ultrastructure of the cell due to the administrations of caffeine and 5-FU. The cells were fixed and stained with uranyl acetate and lead citrate, and observed with JEM-1400 electron microscope (JEOL Ltd, Tokyo, Japan) at 80 kV. The spectra were measured at 24 and 48 h after administration of chemicals. The bands in the region 750 – 950cm$^{-1}$ are assignable to DNA and RNA, and the bands in the region 1200-1300 cm$^{-1}$ a mainly assignable to protein. The spectral changes found in the regions maybe reflect the disintegration of membrane in the cells which was detected by TEM. Thus, results suggest that the effect of caffeine on pancreatic cancer is somewhat similar to the effect of 5-FU in the first 24 h. The normal pancreases cells treated with various concentration of caffeine were observed by Raman spectroscopy at 24 and 48 h. In the PCA score plot, it was described significant dispersion between the control and caffeine treated data groups. After 24 h, the control data set showed an independent group from the treatment data groups. After 48 h, all data sets were overlapped each other and there was no obvious discrimination. The ultrastructural features between the control and treatment groups after 24 h of treatment. At a 100 μg/ml concentration of caffeine, no morphological changes occurred in the normal pancreatic cells.