

Influence of fixation products used in the histological processing in the FTIR spectra of lung cells.

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Numerous factors have been postulated to contribute to the development of lung malign neoplasia in the mammalian. The chemical carcinogens from tobacco are related to be responsible of over 90% of lung tumors around the world. The mortality resulting from this kind of tumor is high because the diagnosis is usually made only in advanced stages of the disease. Therefore, it is necessary to develop new diagnostic methods for detecting the lung neoplastic cells in early stages. Among the optical characterization techniques, the Fourier Transform Infrared spectroscopy (FTIR) can offer high sensibility and accuracy to detect minimal chemical changes into the biological sample and in principle, can be used to differentiate the normal from neoplastic tissues. The aim of the present study is to evaluate the differences on FTIR spectra from the normal cell lines to neoplastic cells, due to different fixation protocols for histological processing. Immortalized, non-cancerous mice lung epithelial cell line E10 (American Type Culture Collection, Rockville, MD, USA) and NNK-transformed E10 cell lines were maintained in complete E10 culture medium (CMRL-1066 (Gibco), 10%SUF and L glutamine 200mM). The cultures were fixed with following substances: 5 % formalin in PBS, metacam (60% methanol, 30% chloroforme and 10% acetyl acid) , 70% alcohol in water, and also the unfixed cells, were cultured and maintained in PBS. The FTIR spectra were acquired on a Nicolet 6700 (Thermo Scientific Nicolet TM, Waltham, MA) spectrophotometer at a 6 cm⁻¹ resolution, 30 scans, in the 4000–500 cm⁻¹ spectral range. Nine infrared spectra were obtained from each fixation protocol. Main region of spectra used in the analysis was 1800-900 cm⁻¹. All spectra were analyzed by vector normalization as well as their 1st and 2nd derivatives, to determine any possible difference in bands and peak position. These results indicate that all fixing protocol change some of the specific characteristics of FTIR spectra. The formalin fixation was the protocol that caused less modification on the spectra related to the unfixed one. Therefore the choice of the fixing protocol depends on the specific information wanted from the spectra. Acknowledgments: FAPESP - CEPID (05/51689-2), CNPq - INFO (573916/2008) and CNPq (143166/2009-3).