FTIR characterization of animal lung cells: normal and precancerous modified e10 cell line

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ABSTRACT

The chemical carcinogens from tobacco are related to over 90% of lung cancers around the world. The risk of death of this kind of cancer is high because the diagnosis usually is made only in advanced stages. Therefore, it is necessary to develop new diagnostic methods for detecting the lung cancer in earlier stages. The Fourier Transform Infrared Spectroscopy (FTIR) can offer high sensibility and accuracy to detect the minimal chemical changes into the biological sample. The aim of this study is to evaluate the differences on infrared spectra between normal lung cells and precancerous lung cells transformed by NNK. Non-cancerous lung cell line e10 (ATCC) and NNK-transformed e10 cell lines were maintained in complete culture medium (1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 [DMEM/Ham's F12], supplemented with 100 ng/ml cholera enterotoxin, 10 lg/ml insulin, 0.5 lg/ml. hydrocortisol, 20 ng/ml epidermal growth factor, and 5% horse serum. The cultures were maintained in alcohol 70%. The infrared spectra were acquired on ATR-FTIR Nicolet 6700 spectrophotometer at 4 cm^{-1} resolution, 30 scans, in the 1800–900 cm^{-1} spectral range. Each sample had 3 spectra recorded, 30 infrared spectra were obtained from each cell line. The second derivate of spectra indicates that there are displacement in 1646 cm^{-1} (amine I) and 1255 cm^{-1}(DNA), allowing the possibility to differentiate the two king of cells, with accuracy of 89,9%. These preliminary results indicate that ATR-FTIR is useful to differentiate normal e10 lung cells from precancerous e10 transformed by NNK.

Keyword list

FTIR, ATR, e10 cell line, lung cancer

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INTRODUCTION

Cancer eradication could be considered one of the greatest challenges of modern medicine since cancer in its various forms is the disease responsible for the majority of human deaths worldwide[1]. According to World Health Organization, one of the most importance strategies to reduce mortality rate is developing more sensitive new diagnostics methods to detect cancer at early stages.

Talking into account all types of cancer, the lung cancer is leading cause of cancer deaths[2] and this kind of cancer is strongly related to smoking habits[3]. despite the enormous advances in lung cancer therapy in the last 30 years, the gain in survival time was only 15% which could be considered unsatisfactory[4]. The FTIR characterization of normal and precancerous lung cells can provide valuable biochemical information for the development of a more sensitive diagnosis method to detect lung cancer at early stages.

Among the infrared spectroscopy techniques, the Attenuated Total Reflectance Infrared spectroscopy (ATR) can offer high sensibility and accuracy to detect small chemical changes into the biological sample with minimal sample preparation [5]. There are several articles which shown that it is possible to identify many changes in the cells on the subcellular level by FTIR – ATR and also that this is a powerful bioanalytical technique for the simultaneous analysis of lipids, proteins, and a variety of organic compounds within the cells[6, 7]. Thus, infrared spectroscopy can be, in principle, used as a tool to study of cell cycle, evaluate and drug effectiveness or the development of diagnostics methods.

GOAL

To evaluate by ATR-FTIR spectroscopy, changes of immortalized non-cancerous human lung epithelial e10 cells and e10 precancerous induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) at a low concentration. The NNK is a carcinogenic substance from tobacco.

MATERIAL AND METHODS

Carcinogenic protocol

The following procedure was performed to obtain chronic carcinogen exposure to the e10 cells. As depicted in Figure 1, 24 hours after every sub-culturing, cultures of e10 cells were treated with NNK at 100 pM for 48 h as one cycle of exposure for 20 cycles; cultures were sub-cultured every 3 days (3 days/cycle). After exposures to NNK(4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone), the survival cells (e10nnk20) was immortalized for future FTIR analysis.

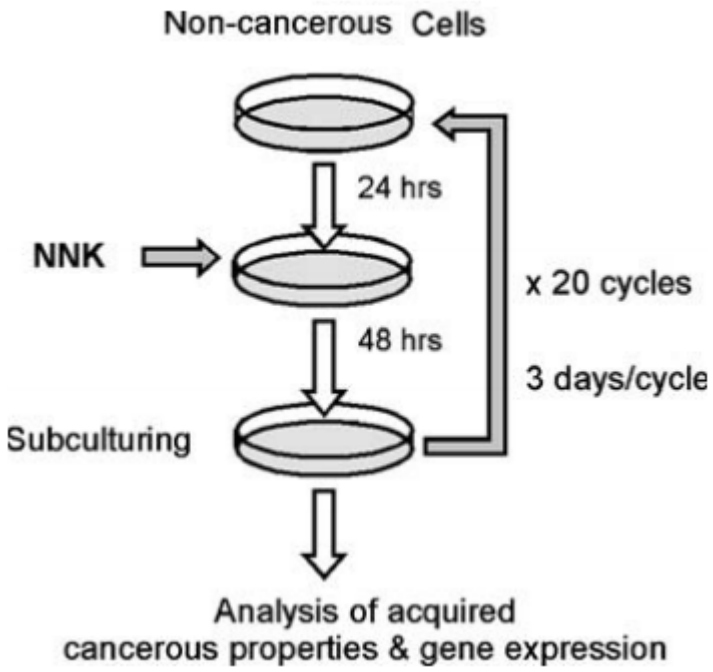

Figure 1: Experimental scheme of carcinogenesis induction[8].

Cell culture

Immortalized mice lung epithelial cell line e10 (American Type Culture Collection, Rockville MD,USA) and e10nnk20 was maintained in complete e10 culture medium (1:1 mixture Dulbecco´s modified Eagle´s medium and Ham´s F12 [DMEM/Ham´s F12], supplemented with 100 ng/ml cholora enterotoxin, 10 µg/ml insulin 0.5 lg/ml hydrocortisol, 20 ng/ml epidermal growth factor, and 5% horse serum) (Life Technologies, Rockville, MD, USA).

Sample preparation

To carry out the experiments, the cells were removed from 6 bottles of each cell culture by using 0.2 mL trypsin-EDTA solution (2.5 g/L; Sigma-Aldrich, St. Louis, MO). They were washed at 1000 rpm/15 minutes with a 0.9% NaCl solution, to remove the growth medium. The cultures were fixed with 70% alcohol. The fixed cells were stored at 5 °C until the FTIR measurement.

FTIR measurements

The spectra were acquired on a FTIR spectrometer (Nicolet 6700, Thermo-Scientific Nicolet TM Waltham, MA) at a 4 cm^{-1} resolution, 32 scans, in the 4000–800 cm^{-1} spectral range. For the ATR-FTIR

measurements, cells were deposited on the diamond crystal (approximately 10 μL with 10^7–10^8 cells) and dried with air for 10 min before the measurements. Five FTIR spectra were acquired from each cell bottle.

Data processing

All FTIR spectra were vector normalized and mean of FTIR spectra and also second derivate of each cell lines was calculated. The mean of second derivate was analyzed to find out the existence of any peak displacement that indicates same differences on the spectra. Principal Component Analysis (PCA) on mean-centered data was used to analyze the data sets to find out if these multivariate techniques are useful used to differentiate between e10 and e10nnk20 cells.

RESULTS AND DISCUSSION.

A typical infrared spectrum of the analyzed cells is presented in figure 2. The cell´s IR spectra shows the mainly absorption band corresponding to proteins, such as amide I (1646-1655cm^{-1}) and amide II (1546-1555 cm^{-1}), DNA (between 1300 cm^{-1} and 900 cm^{-1}). The presence of these biomolecules is well showed with good SNR (signal to noise ratio) and both cells culture have very similar spectral pattern.
The figure 2 shows the mean of IR spectra of e10 (normal cells) and e10nnk20 (abnormal cells).

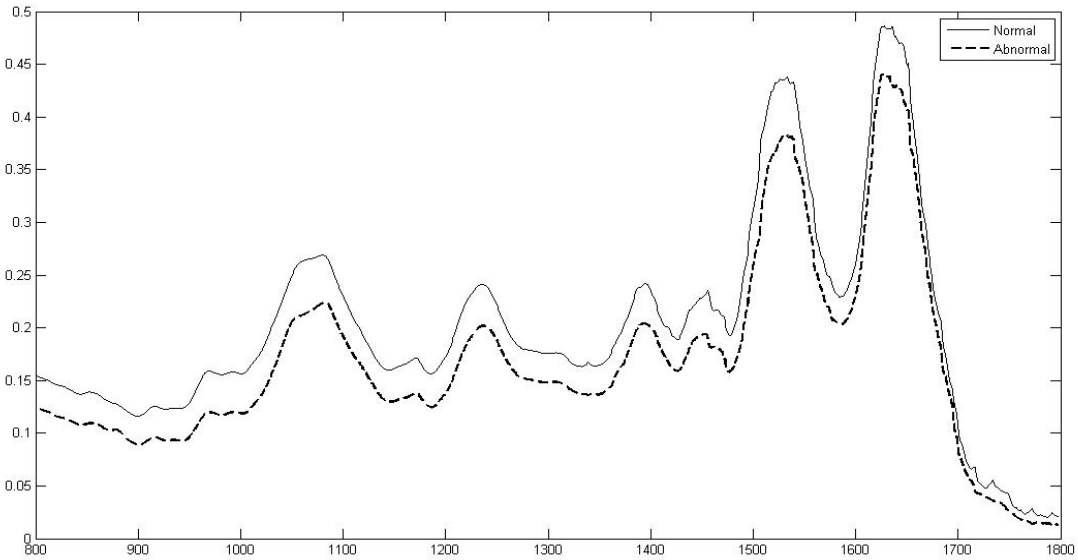

Figure 2: the mean spectra of normal cells and abnormal cells.

These differences of spectra between normal and abnormal cells are better shown in the second derivatives of the spectra. The figure 2 a,b shows the 2 derivatives related a DNA region (900 cm^{-1} – 1300 cm^{-1}) and amide I and II.

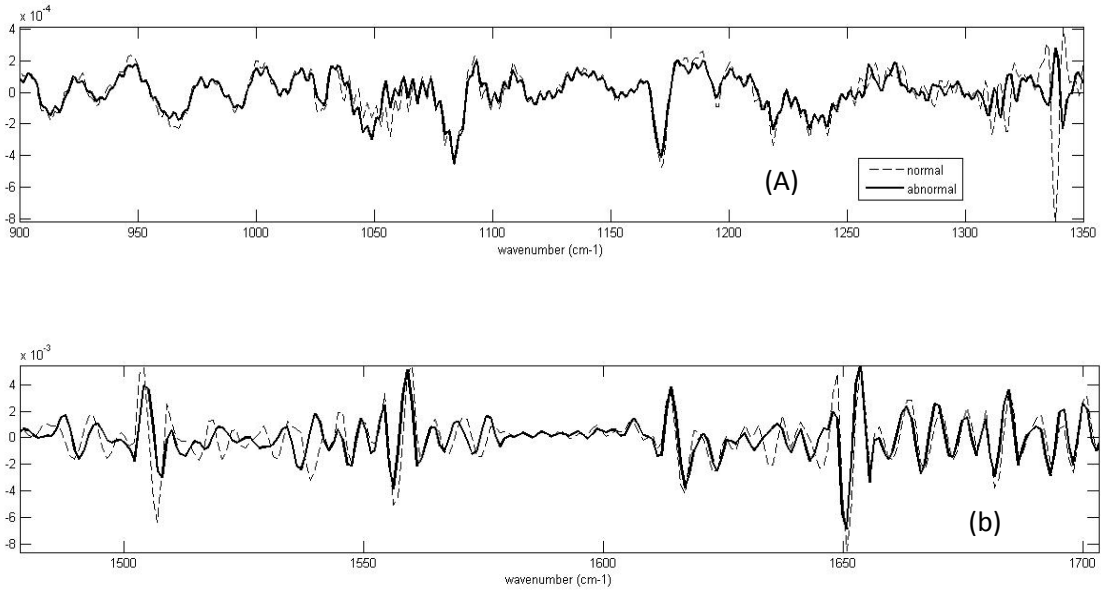

Figure 3: (a) Mean second-derivative spectra of normal and abnormal cells on DNA region. (b) Mean second-derivative spectra of normal and abnormal cells on protein region. Note the displacements in several region of the spectra.

The figure 3 shows several peak displacements. There is a shift (1646 cm^{-1}) on band (1590 cm^{-1} – 1690 cm^{-1}) due to amide I. This region is associated with the conformation of alpha helix structure (1646 cm^{-1}). This displacement is related with protein structures and it can indicate higher proliferation of e10nnk20 than e10 cells. There are studies on the literature that shown the higher proliferation rate on pre-cancerous cells [1, 8].

There is a shift (1255 cm^{-1}) that it is related to PO_2 stretching. Therefore these changes on the spectra into this region are due to DNA structure. Usually the neoplastic culture cells has more binuclear cells and, as a consequence there is a structural changes into the DNA molecule due to its.

The figure 4 show the PCA scores plot of e10 and e10nnk20.

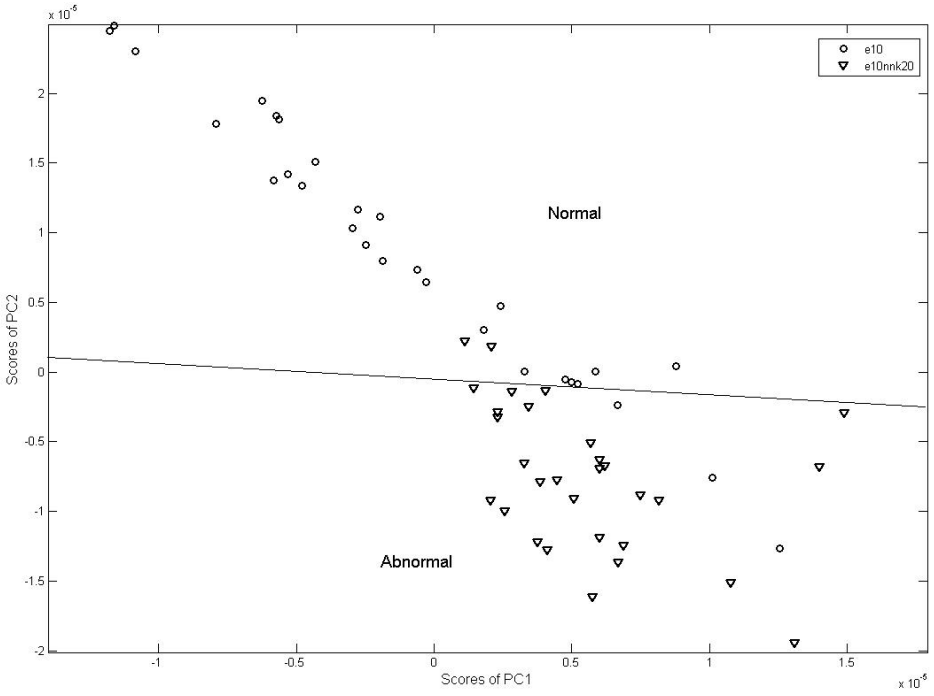

Figure 4: PCA scores plot of e10 and e10nnk20 cells. The line separates normal from abnormal cells.

The figure 4 shows that PCA analysis can be used to differentiate between e10 and e10nnk20 cells lines. There is a normal sample that was removed because the score of PC1 and PC2 were 10 times higher than mean scores of normal group (outlier).

We choose a line that divided the graph into 2 regions. The slope of this line was calculated to increase the sensitivity and specificity. The sensibility founded by this method was 93%, the specificity was 89% and accuracy was 91%. Next step of this work will be increase the number of samples aiming to increase the accuracy of this diagnosis proposal method.

CONCLUSIONS

The results obtained as well the statistical methods applied at FTIR spectra from e10 and e10nnk20 cells shown that it is possible to differentiate normal lung cell from precancerous cells. These results demonstrate that it is possible to develop a new diagnostic method based on FTIR spectroscopy that can be more sensitive for early detection of lung cancer.

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