Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 173 (2017) 809–816

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Fourier transform infra-red spectroscopic signatures for lung cells' epithelial mesenchymal transition: A preliminary report

Atasi Sarkar [a,*], Sanghamitra Sengupta [b], Anirban Mukherjee [c], Jyotirmoy Chatterjee [a]

[a] School of Medical Science and Technology, Indian Institute of Technology Kharagpur, Kharagpur 721302, West Bengal, India
[b] Department of Biochemistry, Calcutta University, Ballygunge, Kolkata 700019, West Bengal, India
[c] Department of Electrical Engineering, Indian Institute of Technology Kharagpur, Kharagpur 721302, West Bengal, India

ARTICLE INFO

Article history:
Received 16 June 2016
Received in revised form 23 September 2016
Accepted 16 October 2016
Available online 17 October 2016

Keyword:
Epithelial mesenchymal transition
FTIR
PCA-LDA
Vector-valued regularized kernel approximation
Forward feature selection

ABSTRACT

Infra red (IR) spectral characterization can provide label-free cellular metabolic signatures of normal and diseased circumstances in a rapid and non-invasive manner. Present study endeavoured to enlist Fourier transform infra red (FTIR) spectroscopic signatures for lung normal and cancer cells during chemically induced epithelial mesenchymal transition (EMT) for which global metabolic dimension is not well reported yet. Occurrence of EMT was validated with morphological and immunocytochemical confirmation. Pre-processed spectral data was analyzed using ANOVA and principal component analysis-linear discriminant analysis (PCA–LDA). Significant differences observed in peak area corresponding to biochemical fingerprint (900–1800 cm^{-1}) and high wave-number (2800–3800 cm^{-1}) regions contributed to adequate PCA–LDA segregation of cells undergoing EMT. The findings were validated by re-analysis of data using another in-house built binary classifier namely vector valued regularized kernel approximation (VVRKFA), in order to understand EMT progression. To improve the classification accuracy, forward feature selection (FFS) tool was employed in extracting potent spectral signatures by eliminating undesirable noise. Gradual increase in classification accuracy with EMT progression of both cell types indicated prominence of the biochemical alterations. Rapid changes in cellular metabolome noted in cancer cells within first 24 h of EMT induction along with higher classification accuracy for cancer cell groups in comparison to normal cells might be attributed to inherent differences between them. Spectral features were suggestive of EMT triggered changes in nucleic acid, protein, lipid and bound water contents which can emerge as the useful markers to capture EMT related cellular characteristics.

1. Introduction

Epithelial mesenchymal transition (EMT) is a critical cellular process during embryogenesis, wound healing, fibrosis and cancer metastasis [1]. It involves switching of epithelial cells into mesenchymal type [2]. Major epithelial features that are altered by EMT include loss of cuboidal morphology, apico–basal organization, cell–cell and cell–matrix interaction, and gain in migratory potential [3]. Altered gene and protein expressions which accompany these changes, have emerged as plausible markers related to EMT and disease progression [4]. Despite its importance in human health and pathology including cancer metastasis, most of the EMT studies are still based on in vitro experiments and yet to be unequivocally appreciated in histopathological practices. Hence, there is a need to evaluate EMT undergoing cells from different perspectives including their local and global label free biochemical signatures.

Global biochemical signatures of cells can be captured by vibration spectroscopy such as Fourier transform infra red (FTIR) [5]. It may provide information on cellular turnover in respect to primary composition including water, proteins, nucleic acids, lipids and carbohydrates [6]. Cellular differentiation/trans-differentiation [7] and physiological deviations [8] triggers shift in molecular vibration modes and alterations in biochemical attributes. Such changes can be documented using infra-red (IR) spectroscopy [8]. Over the last few decades, IR spectroscopy has been proven to be highly sensitive to the conformational changes of biological building blocks in diseased conditions [9] and has offered comparative biochemical profiling of cytological [10], histological [11,12] and bio-fluid [13,14] samples. Information generation using this modality is rapid, non-invasive, label-free and cost-effective [15]. It does not require extensive sample preparation.

Although several spectroscopic studies elaborated biochemical differences between normal and malignancy [16,17] but metastasis associated vital cellular transformation process like EMT is not evaluated from this perspective till date. In this regard, FTIR spectroscopy aided with computational analytics [18] provides valuable options in finding label-free markers having connotation with cellular (normal and

* Corresponding author.
E-mail address: atasi181@gmail.com (A. Sarkar).

http://dx.doi.org/10.1016/j.saa.2016.10.019
1386-1425/

cancer) biochemical status. To inspect biological specimens, the most crucial spectral zones include the fingerprint region (900–1800 cm^{-1}) that extract amide I and II signatures and high wavenumber region (2800–3800 cm^{-1}) which corresponds to stretching vibrations such as S—H, C—H, N—H and O—H molecules [19]. Previously, Zhang et al. elaborated the role of spectral wavenumbers such as 1640, 1550, 1460, 1400, 1240, 1310, 1160 and 1080 cm^{-1} to be useful for discriminating malignant and benign lung tissues [20]. Lee et al. identified nucleic acid related band intensity at 970 and 1085 cm^{-1} which contributed to differentiate normal and cancerous lung epithelial cells [21]. Hence, the present study employs FTIR spectroscopy to evaluate EMT progression in vitro at differing temporal points on lung normal and cancer cells. The critical appreciation of induced EMT thus may be achieved by illustrating discriminatory cellular status at different temporal points and uncovering their probable spectral attributes.

2. Materials and Methods

2.1. Materials

Recombinant human transformation growth factor beta 1 (TGF β1), Dulbecco's modified Eagle's medium (DMEM), Fetal bovine serum (FBS), 1% antibiotics-antimycotic solution were purchased from Himedia (Mumbai, India). 6-diamidino-2-phenylindole (DAPI) and anti-mouse Alexa Fluor 594 were obtained from Invitrogen, Life Technologies, (USA). Primary antibody (mouse monoclonal) against α Smooth Muscle Actin (α SMA) was purchased from Abcam (UK).

2.2. Cell Culture

Lung normal epithelial cell line, L123 [22] and adenocarcinoma cell line, A549, were obtained from National Centre for Cell Science

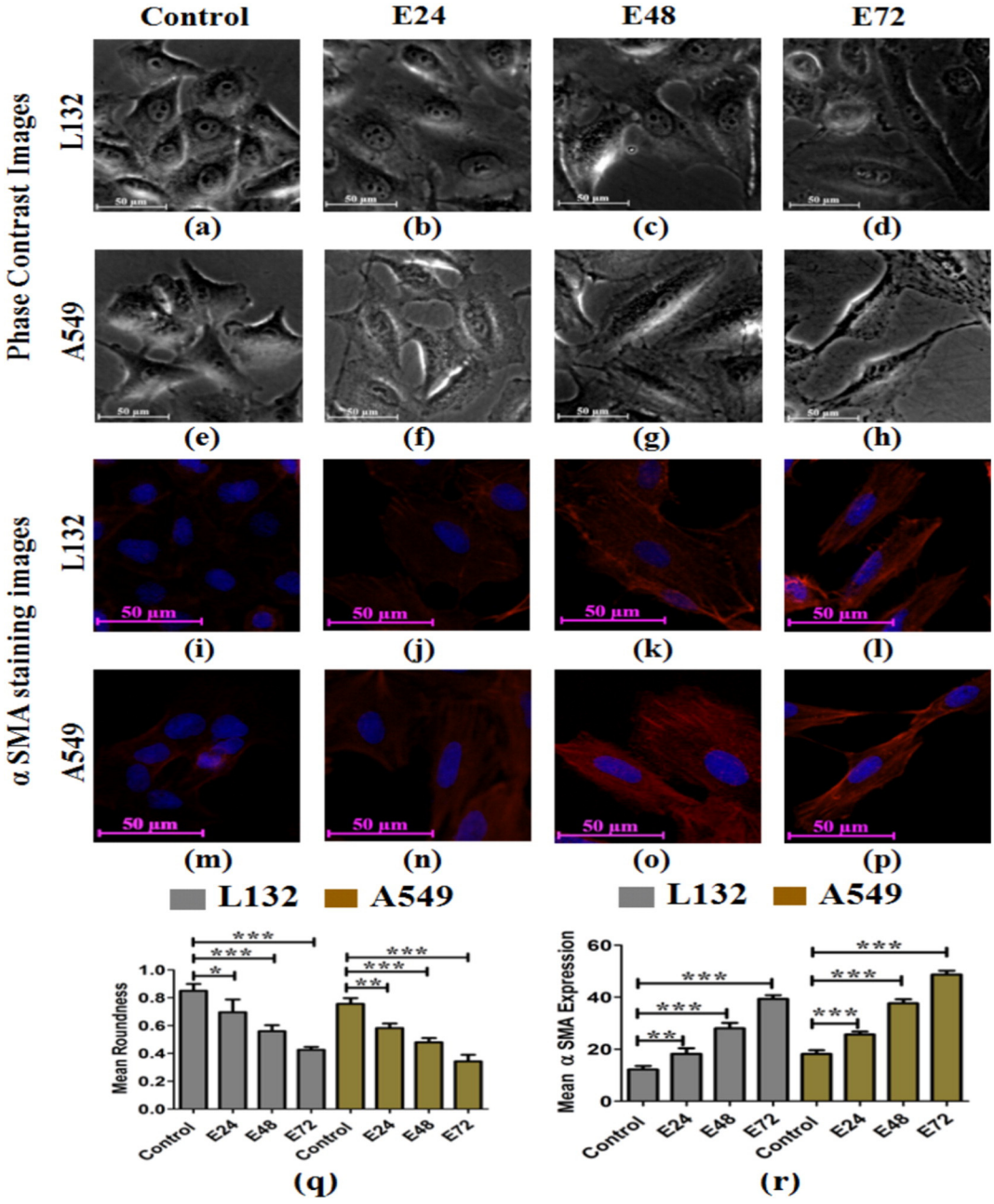

Fig. 1. Phase contrast (a–h) and immunocytochemical images (i–p) showing morphological and cytoskeletal changes respectively in L132 and A549 cells during EMT progression at 200× magnification. Quantification of cellular roundness (q) and α SMA (r) also presented.