MULTIPLEXING OF FOUR ORGANELLE- FLUORESCENT DYES TO MONITOR PHENOTYPE TRANSITIONS OF HUMAN BRONCHIAL SMOOTH MUSCLE CELLS

Salina Othman¹, Nor Shahida Abdul Rahman¹, and Gabriele Ruth Anisah Froemming¹

¹Institute of Medical Molecular Biotechnology, Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh Campus, 47000 Sungai Buloh, Selangor, Malaysia
E-mail: gabi_anisahf@yahoo.com

Key words: BSMC, Hyperplasia, Hypertropy, Multiplexing, COPD, Asthma, Phototoxicity

Background: Bronchial smooth muscle cells (BSMC) play a central role in normal lung physiology by controlling airway contractility. During chronic airway diseases i.e. COPD or asthma BSMC undergo morphological changes which can lead to hyperplasia and hypertropy of the airways. Phenotype switching, from a contractile type to a synthetic/proliferative type, has been reported but not yet demonstrated in live smooth muscle cells in-vitro. The use of fluorescent dyes for the visualization of organelles in living cells and assessment of live/dead cells has an increasing importance in cell biology [¹]. With the introduction of less phototoxic fluorescent dyes for the visualization of organelles in living cells and confocal live cell imaging this task may be possible. Therefore we are aiming to develop a multiplexing method that will allow to observe and measure the transition from one phenotype to another.

Objectives: To develop a multiplex cell staining protocol for BSMC that allows studying phenotype and cell morphology changes under prolonged serum deprivation.

Methods: BSMC were seeded at subconfluent density in two-well chamber slides and after reaching confluence, the cells were switched to serum-free medium and observed for up to 15 days. Then, the cells were fixed with 4% Paraformaldehyde for 24 hours, labelled with DAPI, MitoTracker® Red CMXRos, Alexa Fluor 635 Phalloidin and Anti-alpha-tubulin-FITC and mounted with ProLong® Gold Antifade Reagent. Finally the cells were observed under Confocal Laser Scanning Microscope and the images were analyzed using Leica QWIN Software.

Results & Conclusion: After 8 days of serum deprivation, BSMC were starting to show two distinct groups of cells: 1) spindle-shaped elongated cells which showed large amount of smooth muscle contractile protein, and 2) flat and circular cells which showed little. The first group showed decreased cell size and a decrease in abundance of synthetic organelles whereas the second group showed an increase in cell size and abundance of synthetic organelles. Centrally located, elongated nuclei were identified in the spindle-shaped, elongated cells, while round nuclei were seen in the flat and circular cells. The image analysis showed that the intensity of Alexa Fluor 635 Phalloidin and Anti-alpha-tubulin-FITC was higher in the first group of cells compared to the second group and the intensity of MitoTracker® Red CMXRos was higher in the second group of cells compared to the first group. In conclusion, serum deprivation of bronchial smooth muscle cells lead to a phenotype switch after 8 days and the used four-color fluorescence confocal method allowed not only to distinguish but also to quantitate the changes between the two phenotypes. However measuring these changes in live cells requires more optimisations and new imaging techniques to avoid phototoxicity induced changes.

References;