Fourier transform infrared microspectroscopy identifies early lineage commitment in differentiating human embryonic stem cells (hESCs)

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This research is concerned with the goal of directing the differentiation of hESCs towards specific lineages of interest. For example, it may be possible to provide normal cells differentiated from hESCs to replace those damaged through disease processes. It is anticipated that cells prepared for clinical use will need to be differentiated towards the lineage of interest in order to maximise the chance of the efficacy of treatment. Therefore, it will be necessary to have accurate and reproducible assays that could be rapidly performed to validate the efficacy and safety of the hESC differentiation process and to 'certify' each batch of differentiated cells prior to transplantation.

To determine whether FT-IR spectroscopy could be used to classify early stages of hESCs differentiation, three experiments were conducted. In each experiment, analyses were performed on replicate cultures of undifferentiated hESCs and hESCs differentiated towards ectodermal lineages by culture in serum free medium supplemented with FGF2 or towards mesendodermal precursors by a combination of BMP4 and Activin A [1,2].

Cells were cytospun to produce monolayers of cells on IR reflective glass slides and dried under light vacuum in a desiccator. Infrared images acquired with a Varian focal plane array (FPA) microspectrometer. Spectra extracted from the FPA images were pre-processed using a second derivative and normalized using Extended Multiplicative Signal Correction. Partial Least Squares [3] modelling was used to examine the variability of all the FT-IR spectra data sets acquired from the different cell populations sampled in each experiment. Distinct clustering of spectra from the three treatment groups is most clearly visualised in PC1 versus PC2 versus PC3 scores plots from the PLS modelling. hESC spectra were separated from the other spectral groups along PC1, whereas BMP4/Activin A and FGF2 spectra were clustered separately along PC3. Regression loadings plots indicate the spectral bands showing the greatest variation and hence most responsible for the clustering observed in the scores plots. PC1 and PC3 loadings were very similar between all three experiments and indicated differences in bands associated with lipids, proteins, nucleic acids and carbohydrates.

Two different types of classification were compared in their ability to discriminate spectra drawn from the three differentiation classes using independent validation sets: Partial Least Squares Discriminant Analysis [3] and Artificial Neural Network (ANN) analysis [4]. Both performed equally well with better than 97% of spectra from independent validation sets correctly classified into each of the assigned classes in all experiments.

We are now repeating the experiment with live cells using a purpose-built IR wet cell and employing a synchrotron source of IR light to target single cells. Differences between live and dried hESCs spectra will be discussed as well as accuracy of classification based on the two sample types will be shown.

References:

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