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Monitoring of embryonic stem cell differentiation trajectories by intact cell mass spectrometry

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1. Overview

A robust, feasible and unbiased method was introduced to monitor cell culture quality control and follow differentiation of human embryonic stem cells (hESCs). Discrimination of mass spectrum fingerprints by cluster analysis clearly reveals the functional as well as undesired or hazardous cell phenotypes.

2. Introduction

Human embryonic stem cells (hESCs) are promising tools for disease-modeling, cell therapy, bio-industry or drug development. However, long-term cultured hESCs finally develop hidden phenotypic changes, cumulatively acquire various alterations on both the genetic and non-genetic levels and despite advanced culture techniques, the culture-adapted clones with unwanted properties clones are inevitably selected. However, these changes could remain unnoticed until they alter the genome, karyotype or cell phenotype, even in case of the high expression of stemness-associated transcription factors, or their differentiation capacity, or a typical morphology. Furthermore, molecular, genetic, and/or light-microscopy analyses can fail in the case of the genetically or karyotypically silent changes that are evoked in cultured cells. Thus, recent quality control approaches often suffer of low sensitivity or may produce biased output. Therefore, there is an ongoing need for sensitive, robust, feasible and affordable methods revealing abnormalities in cell phenotype.

3. Methods

hESCs were cultured under identical conditions, and processed immediately after harvesting for intact-cell MS. Harvested cells were washed in phosphate-buffered saline, counted, resuspended in the isotonic ammonium bicarbonate buffer, mixed with an acidified matrix containing sinapinic and 2,2,2-trifluoroacetic acid, and applied to a target plate. The mass spectra were recorded on MALDI-8020 and verified on Axima CFR (both Shimadzu, Manchester, UK) in linear positive ion mode over the 2,000–20,000 m/z range. Data sets were exported in ASCII. The final normalized spectral dataset containing selected m/z values with assigned peak intensities was subjected to statistical analyses.

4. Results

4-1. Proof of principle

We compared mass spectra of hESCs cultured for varying time that developed distinct karyotypic or molecular traits (Experimental scenario 1). The final normalized spectral dataset was subjected to statistical analyses. Using PCA, the correctly clustered populations corresponding to short and long time of culture were clearly identified. For details see Vanhara et al. 2018.

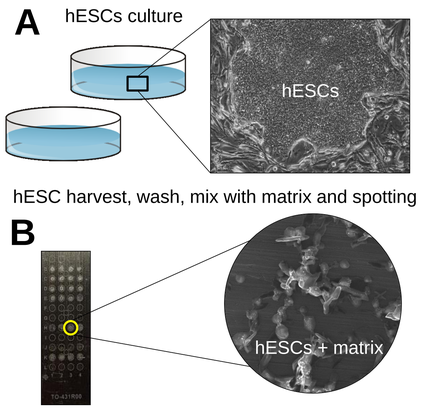


Figure 1 Experimental workflow. hESCs growing in colonies on a feeder layer of mouse embryonic fibroblast (A) are cultured under standard conditions, then harvested, washed in isotonic, MS-compatible buffers and spotted in a mixture with the matrix (TFA-acidified sinapinic acid) directly on the target plate. Scanning electron microphotograph (SEM) of a target spot documenting that cells keep their integrity after spotting (B). Mass spectra were recorded on Shimadzu MALDI TOF instruments AXIMA CFR and MALDI-8020 (C).

Discrimination of early and culture-adapted hESCs

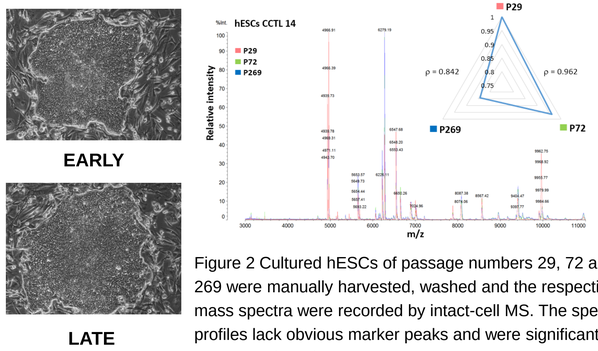


Figure 2 Cultured hESCs of passage numbers 29, 72 and 269 were manually harvested, washed and the respective mass spectra were recorded by intact-cell MS. The spectral profiles lack obvious marker peaks and were significantly correlated (inset).

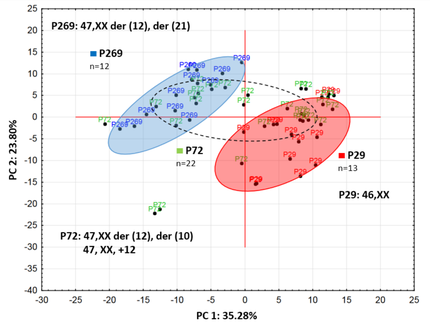


Figure 3 PCA of spectral datasets discriminates between individual cohorts of hESCs. Each point in the PCA plot represents a unique biological sample. While the morphology of hESCs remains unaltered over the time in culture, hidden karyotype alterations develop.

3-2. Monitoring of differentiation of early lung progenitors

Next, shifts were monitored in the hESC phenotypes after induction of differentiation towards early lung progenitors (ELEPs). Mass spectra recorded from stimulated and control cells reflecting the metabolomic profile between 2000 – 20000 Da allowed discrimination by principal component analysis (PCA) and monitoring of the differentiation process towards desired cell type.

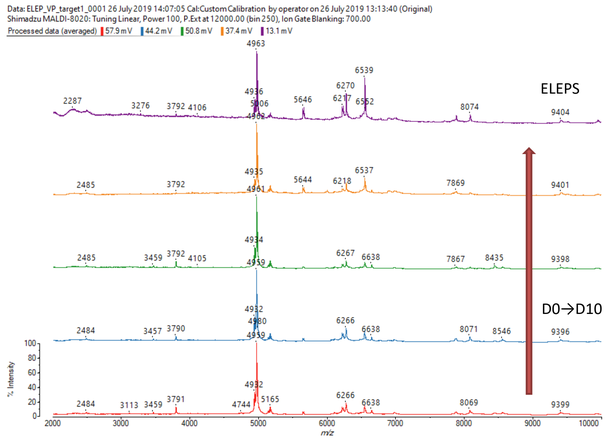
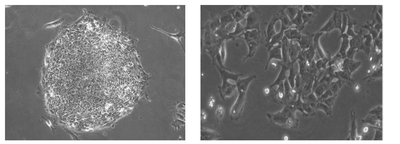
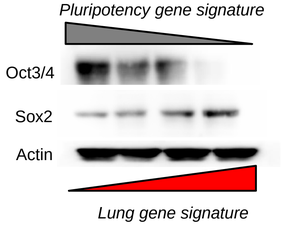
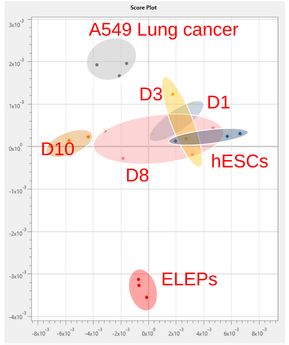


Figure 4 Mass spectra documenting patterns of various low-mass molecular entities. hESCs were stimulated for differentiation towards ELEPs, harvested at indicated time intervals and processed for intact-cell MALDI TOF MS as described in Figure 1.

Monitoring of differentiation trajectory from hESCs to early lung progenitors







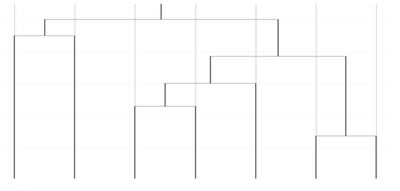


Figure 5 hESCs stimulated for differentiation towards early lung progenitors (ELEPs) were harvested at indicated time intervals and analyzed by intact-cell MS.
Mass spectra recorded from differentiating hESCs → ELEPs contained sufficient information to discriminate individual differentiation stages. We documented differentiation series from hESCs through D1-D10 cells to ELEPs, based solely on changes in mass spectrum profile, where ELEPs represented a distinct cell entity in differentiation route. A549 lung cancer cell line was used as a phenotypically distant, but still lung-associated pathological control. Each point in the plot represents a unique mass spectrum.

5. Conclusions

Intact-cell MALDI TOF MS can discriminate minute changes occurring in otherwise identical cells and provide a highly sensitive and feasible tool for monitoring of bio-industrial or clinical-grade routine cultures of hESCs

6. Acknowledgements

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7. References

Vanhara, P., L. Kucera, L. Prokes, L. Jureckova, E. M. Pena-Mendez, J. Havel and A. Hampl (2018). "Intact Cell Mass Spectrometry as a Quality Control Tool for Revealing Minute Phenotypic Changes of Cultured Human Embryonic Stem Cells." Stem Cells Transl Med 7(1): 109-114.