Intact-Cell Mass Spectrometry for Monitoring of Stem Cells Cultures

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INTRODUCTION

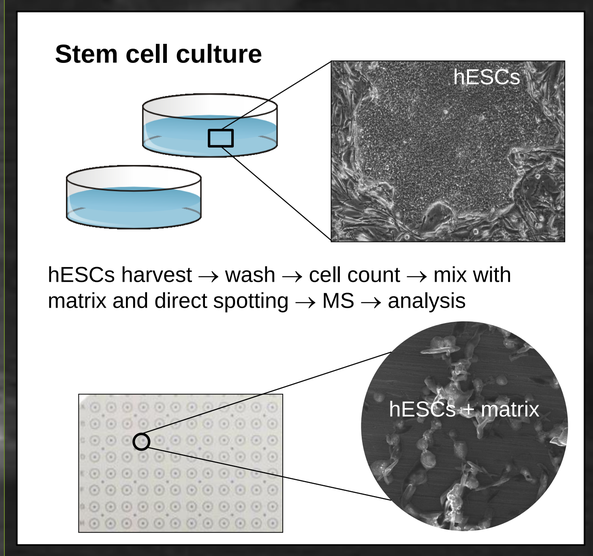
- Human embryonic stem cells (hESCs) represent a promising tool for cell therapy, bio-industry or drug development. However, long-term cultured hESCs finally develop hidden phenotypic changes, cumulatively acquire various alterations on both the genetic and non-genetic levels and despite advanced culture techniques, the culture-adapted clones with unwanted properties 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 a biased output.
- hESCs differentiation to clinically relevant cell types is a gradual proces, where final phenotype is achieved. However, the substantial heterogeneity in the differentiation process may produce aberrant cells with unwanted properties, such as lack of functional phenotype, or propensity to cancer growth. Early lung progenitors (ELEPs) are direct precursors of mature lung cells (pneumocytes) that differentiate from hESCs under specific conditions through several stages (D1-D10). The differentiation pathway can be outlined specifically by molecular markers, but an unbiased, sensitive and robust tool for discrimination of ELEPs from pluripotent or transitional stages is still missing.

Here we show that intact-cell MS can discriminate normal and aberrant hESCs, as well as verify phenotype of differentiated early lung progenitors.

RESULTS

- First, we compared mass spectra of hESCs cultured for varying time that developed distinct aberrant karyotypic or molecular traits. 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. Next, we monitored changes in spectral profiles of hESCs after induction of differentiation towards early lung progenitors (ELEPs). Mass spectra recorded from differentiating and control cells reflecting the metabolomic profile between 2000-20000 Da allowed discrimination by cluster analysis, and monitoring of the differentiation process.

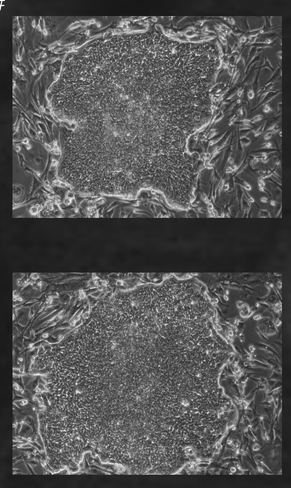
EXPERIMENTAL WORKFLOW

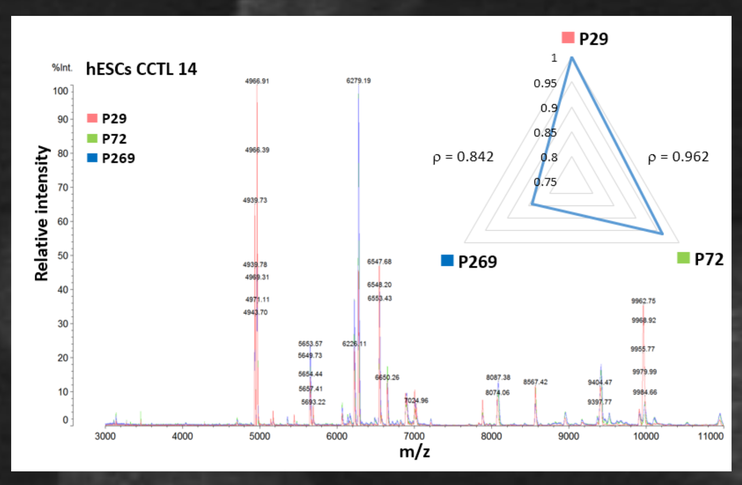


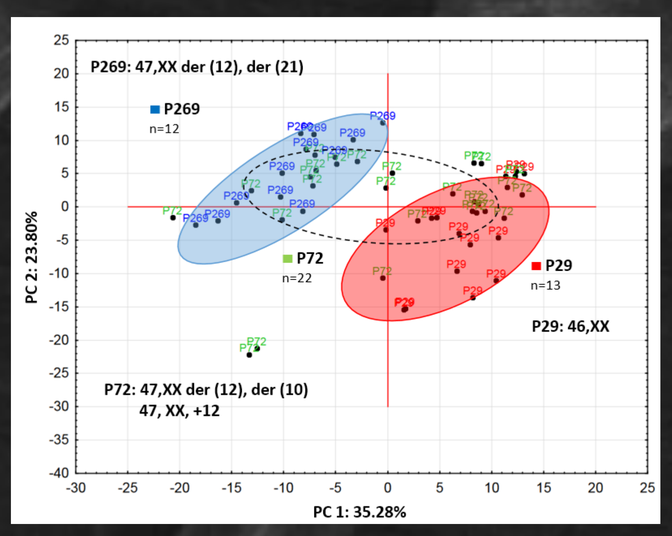
- Human embryonic stem cells (hESCs) growing in colonies were cultured under standard conditions, harvested, washed in isotonic, MS-compatible buffers and spotted in a mixture with the acidified matrix directly on the target plate.
- Scanning electron microphotograph (SEM) of a cell samle on the target spot, documenting that cells keep their integrity after spotting.
- For experimental details see [1] or:

PROOF OF PRINCIPLE

DISCRIMINATION OF NORMAL AND ABERRANT STEM CELLS







- Cultured human embryonic stem cells (hESCs CCTL 14) of passage numbers 29 (early, normal), 72 and 269 (late, aberrant) were manually harvested, washed and processed by intact-cell MS. The spectral profiles lacked obvious marker peaks and were significantly correlated (inset).
- While the morphology of hESCs remained unaltered over the time in culture, hidden karyotype alterations developed.
- PCA of spectral datasets discriminated between individual cohorts of hESCs. Each point in the PCA plot represents a unique biological sample [1].

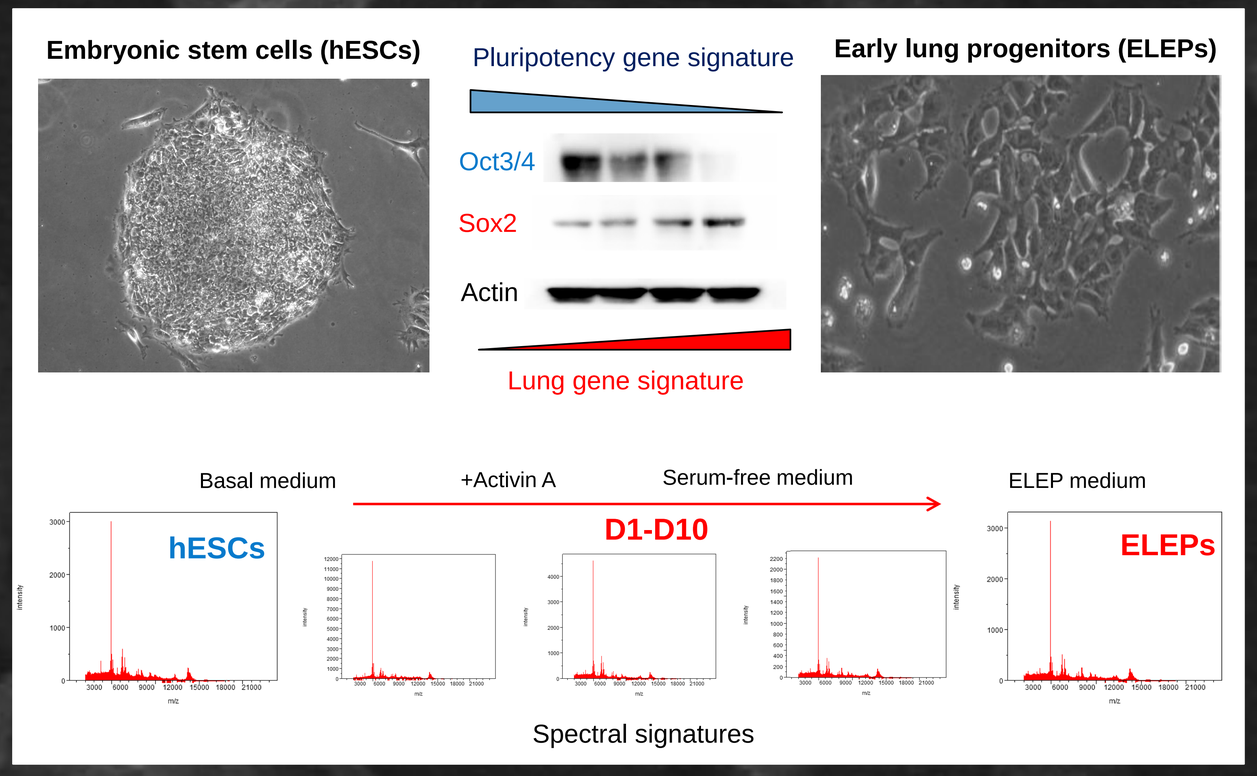
In summary, spectral fingerprints discriminate aberrant from normal hESCs.

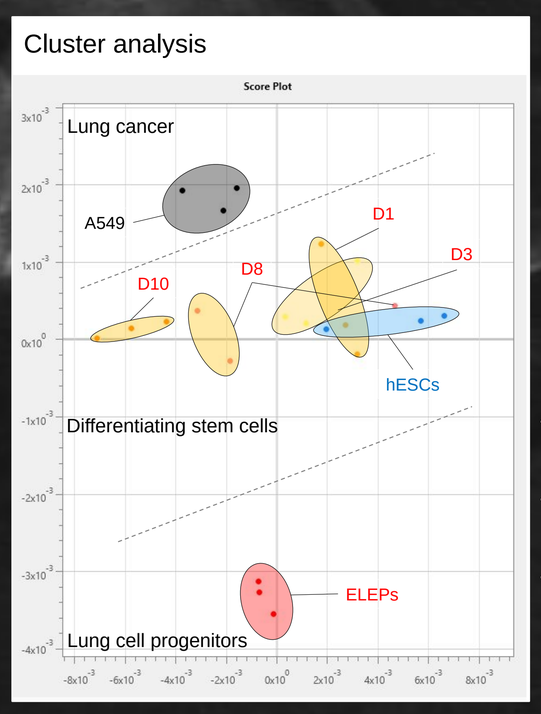
CONCLUSIONS

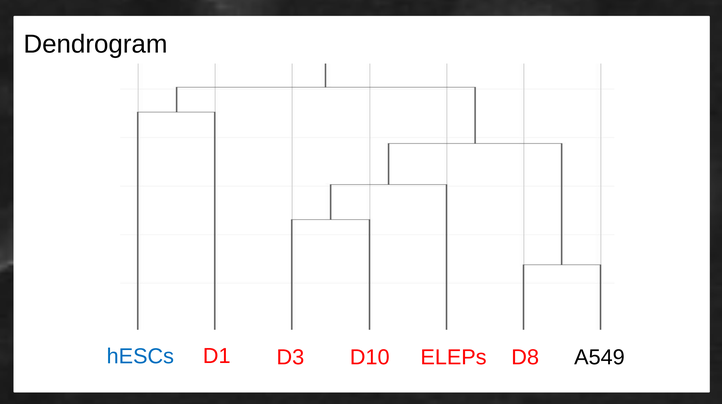
Intact-cell MS discriminates minute changes occurring in otherwise identical cells, and provide a highly sensitive and feasible tool for monitoring of bio-industrial or clinical-grade routine cultures of pluripotent stem cells and their derivatives.

PRECLINICAL APPLICATION

MONITORING OF DIFFERENTIATION TOWARDS EARLY LUNG PROGENITORS







- hESCs stimulated for differentiation towards early lung progenitors 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 control. Each point in the plot represents a unique mass spectrum.

Spectral fingerprints discriminate early lung progenitors from pluripotent and cancer cells.

REFERENCES

[1] Vaňhara P, Kučera L, Prokeš L, Jurečková L, Peña-Méndez EM, Havel J, Hampl A. Intact Cell Mass Spectrometry as a Quality Control Tool for Revealing Minute Phenotypic Changes of Cultured Human Embryonic Stem Cells. Stem Cells Transl Med. 2018. 7(1):109-114. doi: 10.1002/sctm.17-0107.

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