Intact-cell Mass Spectrometry for Pluripotent Stem Cell Identification and Authentication

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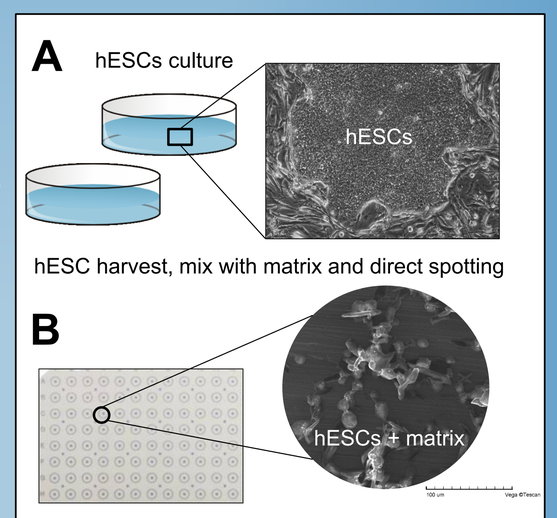
INTRODUCTION

Human embryonic stem cells (hESCs) are able to self-renew, and in parallel to differentiate into all cell types of human body. As such, they are 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 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, e.g. c-Myc, Sox-2, Klf4, Nanog, or Oct3/4, 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 suffers 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**.



Experimental workflow. hESCs growing in colonies (**A**) are cultured under standard conditions, harvested, washed in isotonic, MS-compatible buffers and spotted in a mixture with the matrix directly on the target plate. Scanning electron microphotograph (SEM) of a target spot documenting that cells keep their integrity after spotting (**B**).

METHODS

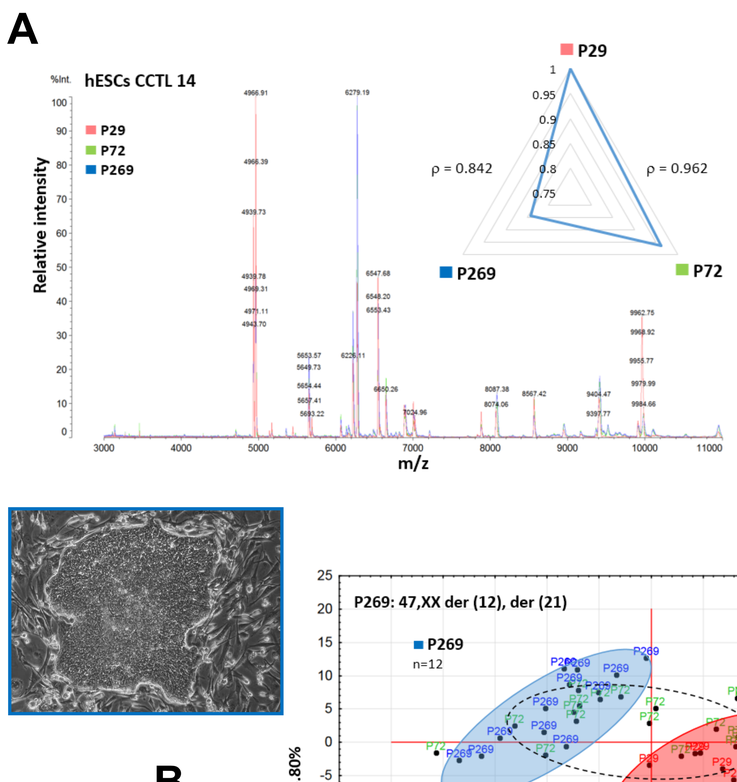
We modeled shifts in CCTL-14 hESC line using different cell culture strategies and addressed changes in cell status by intact-cell MALDI-TOF mass spectrometry (MS). To avoid experimental bias and to make the experimenting feasible and as close to routine as possible, we collected the intact hESCs, washed them in phosphate-buffered saline and counted. Finally, the cells were resuspended in 150 mM ammonium bicarbonate buffer to the final concentration of 10 × 10^6 cells per ml. The cell suspension was then mixed with an acidified matrix containing sinapinic acid and 2,2,2-trifluoroacetic acid in a 2:1 sample to matrix ratio and applied to a target plate. The mass spectra were recorded in linear positive ion mode using an AXIMA-CFR mass spectrometer from Shimadzu Biotech (Kratos, UK) equipped with a nitrogen laser (337 nm). The mass spectra were normalized and analyzed by Pearson's correlation, principal component analysis (PCA) and artificial neural networks (ANN).

RESULTS

hESCs were cultured under identical laboratory conditions, harvested 48 hours after seeding, and processed immediately for mass spectrometry. Then 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. Next, we were curious if we are able to determine shifts in the hESC phenotypes after induction of differentiation. We stimulated hESCs shortly to induce neural differentiation and performed MS analysis (**Experimental scenario 2**). We found that the mass spectra from stimulated and control cells reflecting the metabolomic profile were highly similar and lacked obvious specific peaks that would allow a direct search for biomarkers. Also the PCA only partially discriminated the untreated and differentiating hESCs. Using the artificial intelligence tools, the correctly trained ANN correctly assigned the unknown spectrum to the appropriate sample group and revealed hidden differences in cell phenotype.

EXPERIMENTAL SCENARIO 1

DISCRIMINATION OF PRISTINE AND CULTURE-ADAPTED hESCs

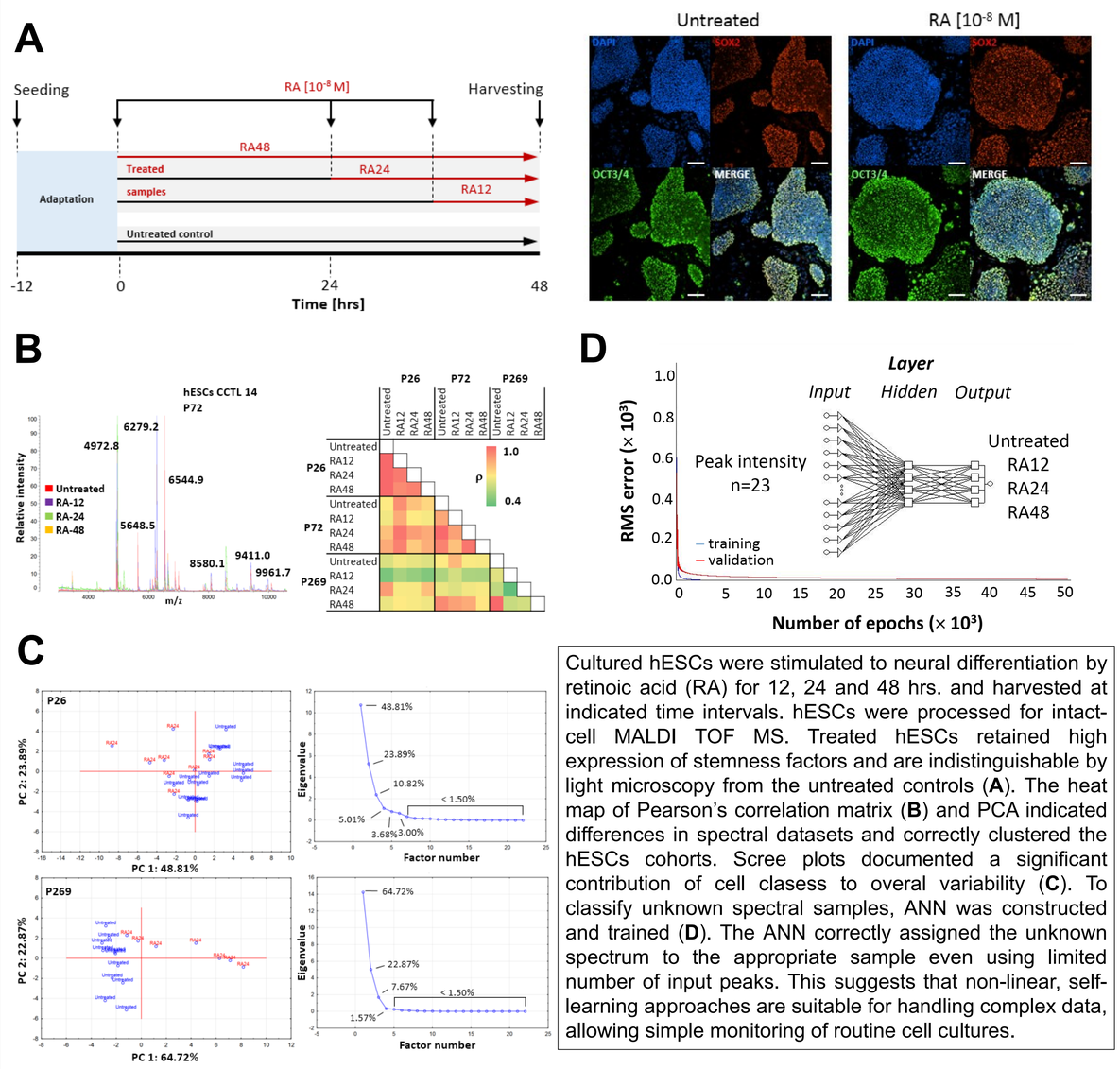


Cultured hESCs of passage numbers 29, 72 and 269 were manually harvested, washed and the respective mass spectra were recorded by intact-cell MALDI TOF MS (**A**). The spectral profile lacked any obvious marker peaks and were significantly correlated (**inset**).

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

EXPERIMENTAL SCENARIO 2

DISCRIMINATION OF hESCs COMMITTED FOR DIFFERENTIATION



Cultured hESCs were stimulated to neural differentiation by retinoic acid (RA) for 12, 24 and 48 hrs. and harvested at indicated time intervals. hESCs were processed for intact-cell MALDI TOF MS. Treated hESCs retained high expression of stemness factors and are indistinguishable by light microscopy from the untreated controls (**A**). The heat map of Pearson's correlation matrix (**B**) and PCA indicated differences in spectral datasets and correctly clustered the hESCs cohorts. Scree plots documented a significant contribution of cell clasess to overal variability (**C**). To classify unknown spectral samples, ANN was constructed and trained (**D**). The ANN correctly assigned the unknown spectrum to the appropriate sample even using limited number of input peaks. This suggests that non-linear, self-learning approaches are suitable for handling complex data, allowing simple monitoring of routine cell cultures.

DISCUSSION

The stability of stem cell cultures is an essential precondition for applications in which the cell product variability, culture cross-contamination, batch consistency, or phenotype stability are critical. In our previous work (1), we show, that ANN-coupled mass spectrometry is capable to distinguish cell types in binary mixtures both qualitatively and quantitatively, or reflect molecular changes in cultured cells. Here, we present that intact-cell MS (MALDI-TOF MS) and ANN analysis can reveal the hidden variability in hESCs. This also suggests that non-linear, statistical and artificial intelligence approaches, can handle complex biological data reflecting minute changes in metabolic states. In summary, we introduce a feasible tool for MS-based monitoring of clinical-grade routine cultures of pluripotent stem cells (2).

CONCLUSIONS

Intact-cell MALDI TOF MS can discriminate minute changes occuring in otherwise identical cells and provide a highly sensitive and feasible tool for clinical-grade cultures of hESCs.

REFERENCES

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2. 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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