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Intact-cell MALDI TOF mass spectrometry for monitoring of stem cell cultures

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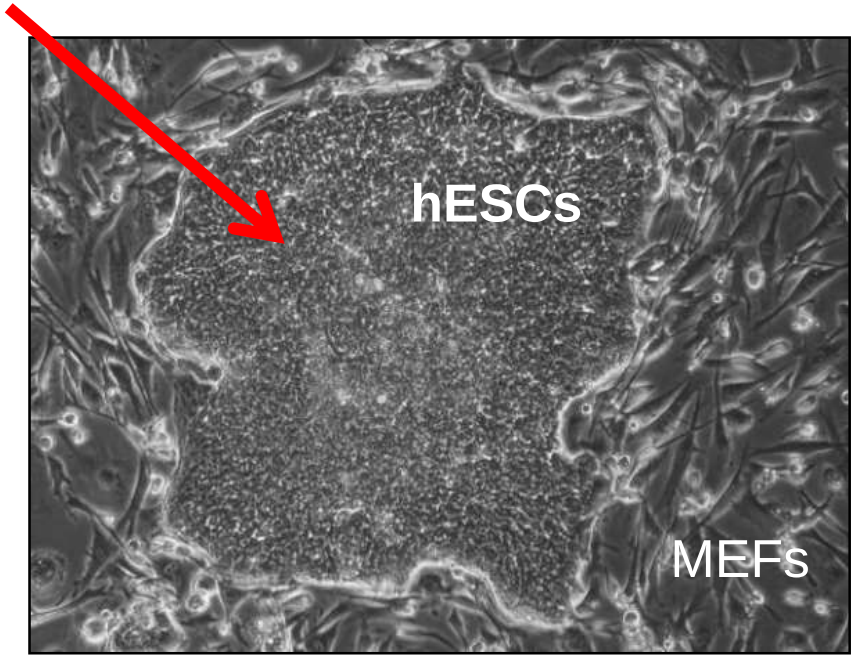
Human embryonic stem cells (hESCs)

- embryoblast of pre-implantation blastocyst
- pluripotency
- capable of infinite self-renew *in vitro*
- source for cell-based therapy, regenerative medicine and tissue engineering

- 2003: derivation of own hESCs lines by P. Dvorak P and A. Hampl



... raised hopes and expectations

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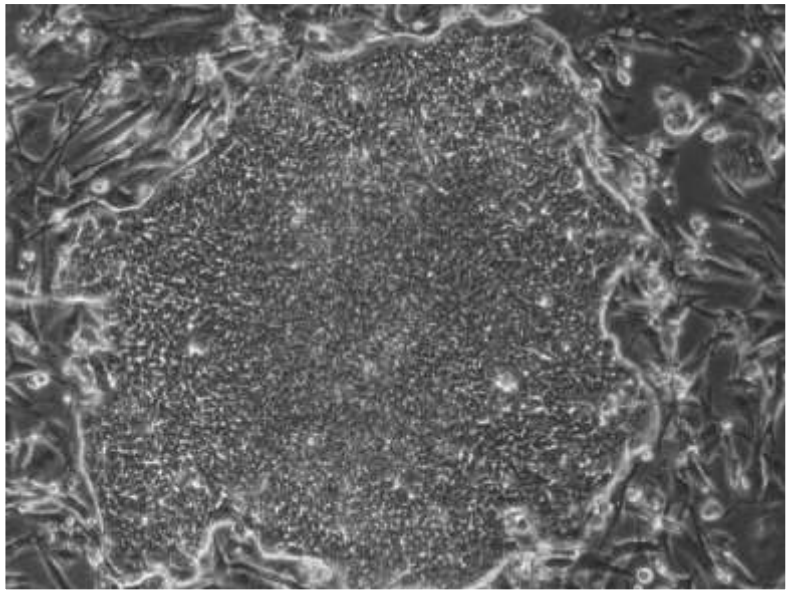
Long-term hESCs cultures produce aberrant hESCs



TIME IN CULTURE

- chromosomal aberrations
- alterations of epigenomic landscape
- gene expression and phenotype shifts

- failure to differentiate
- cancer development
- immune rejection

Development of aberrant cells in culture ...
... applications in clinics are still hazardous

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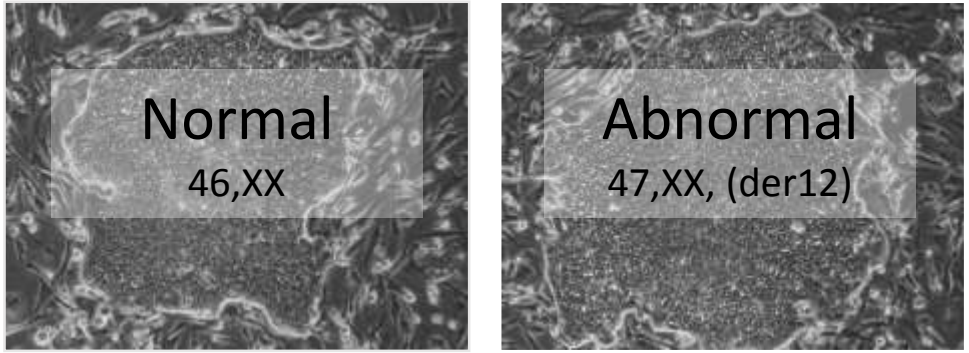
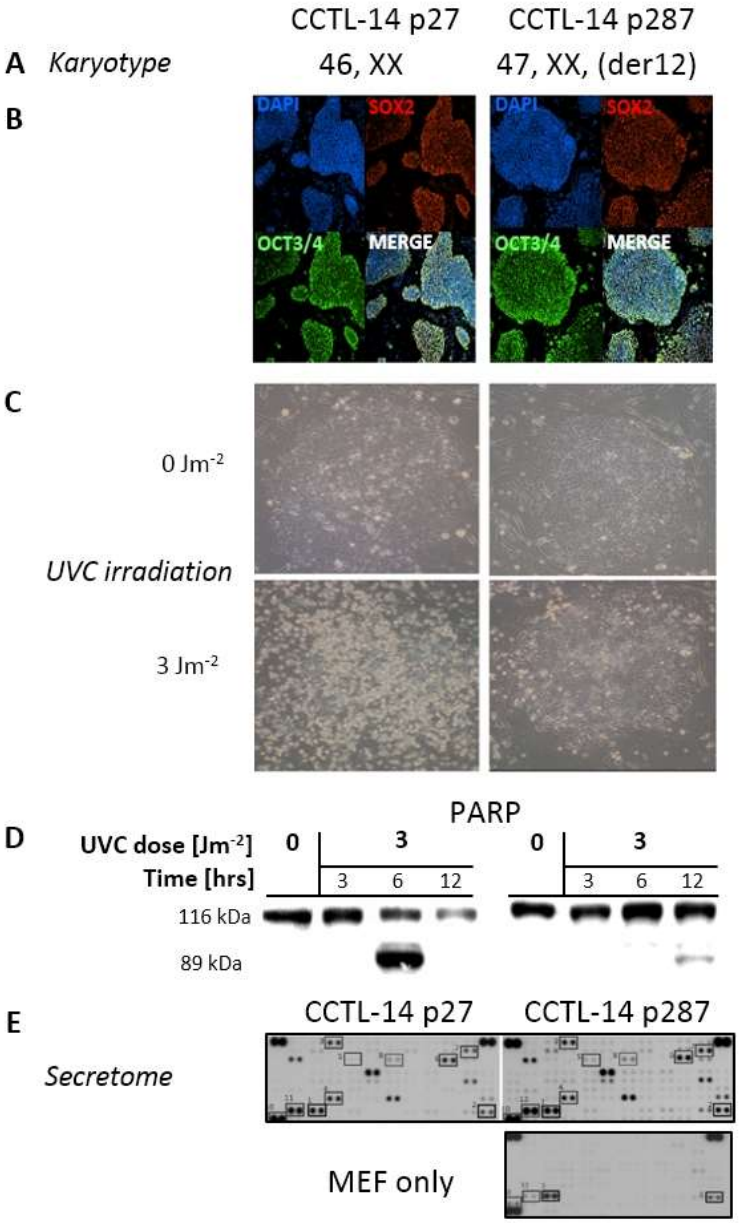
Identification of aberrant hESCs

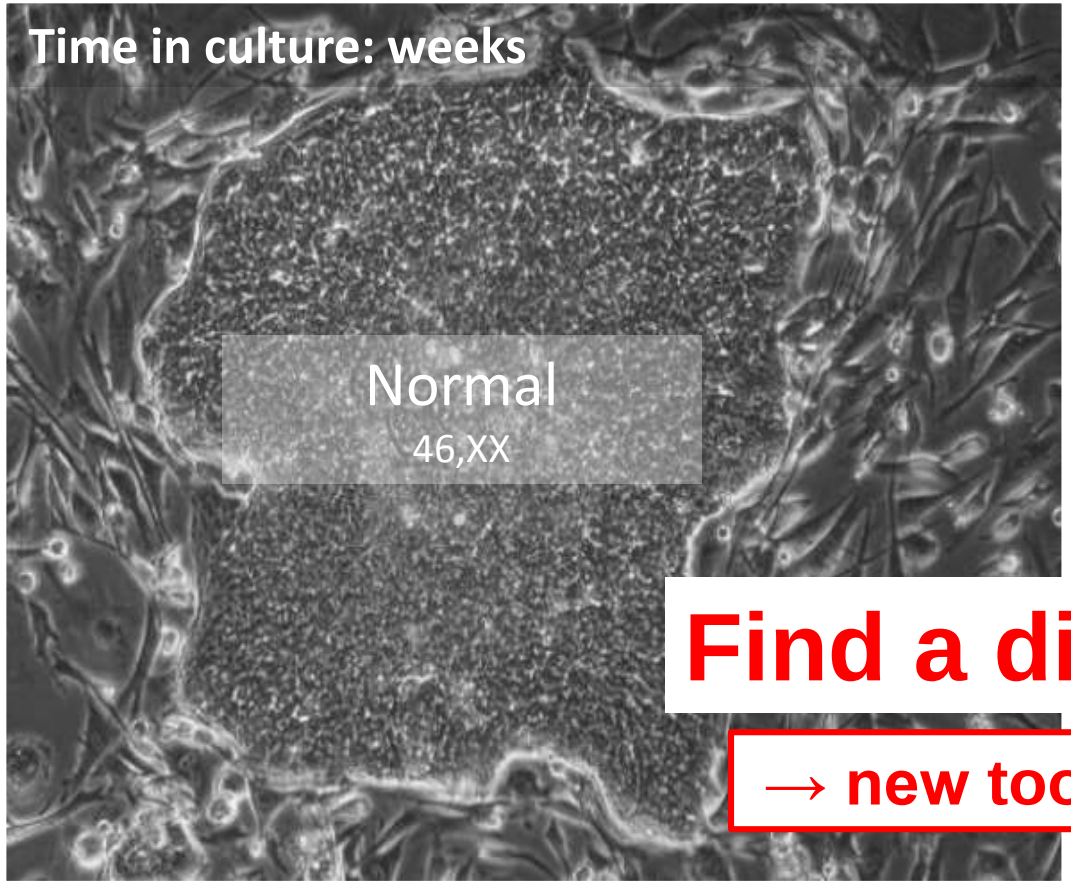


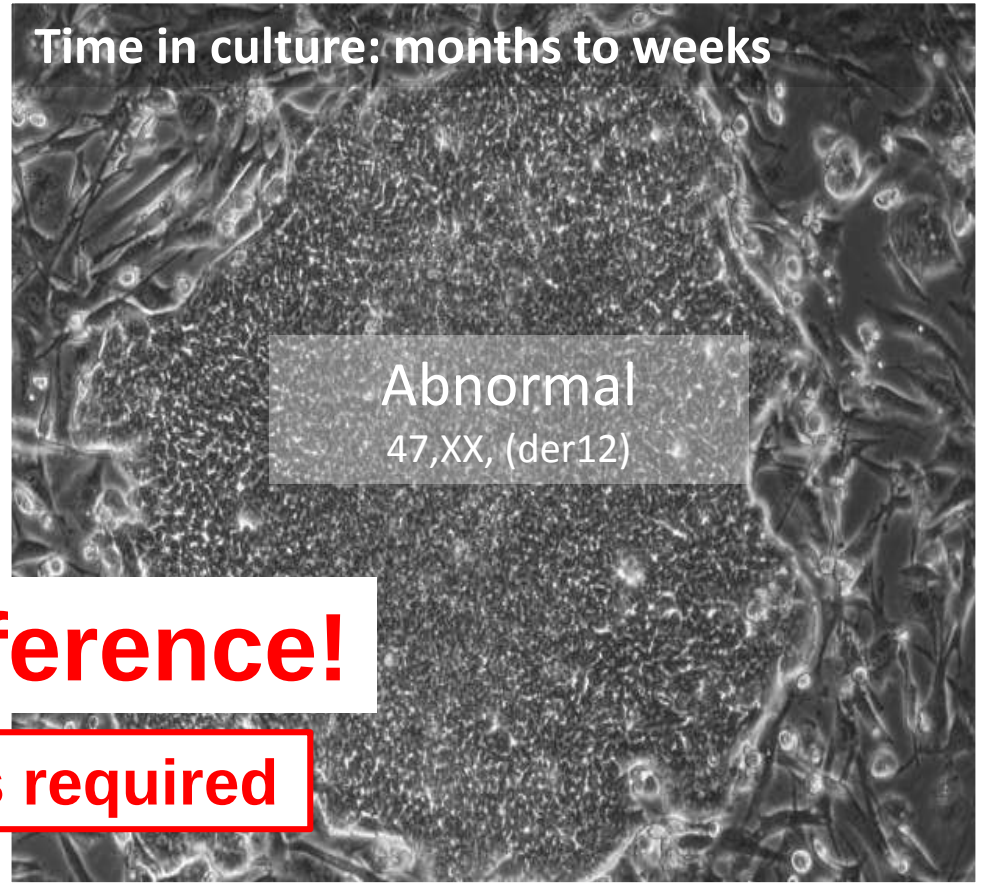
- deregulation of proliferation
- production of cytokines and immunomodulators
- resistence to cell death
- differentiation shifts
- cancer-prone phenotype



Identification of aberrant hESCs

- at global levels, morphologically and genetically uniform
- high expression of stemness factors
- visual, molecular or STR authentication fails





Find a difference!

→ new tools required

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Intact-cell MALDI TOF MS

- robust, sensitive, feasible, affordable
- no extraction, fractionation, or compound isolation
- full mass spectrum reflects chemical composition of cells
- similar concept to bacterial biotyping

Hypothesis: Mass spectrum reflects unique chemical composition of highly complex biological samples and thus can provide effective tool for cell technology and clinical applications

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Intact-cell MALDI TOF MS

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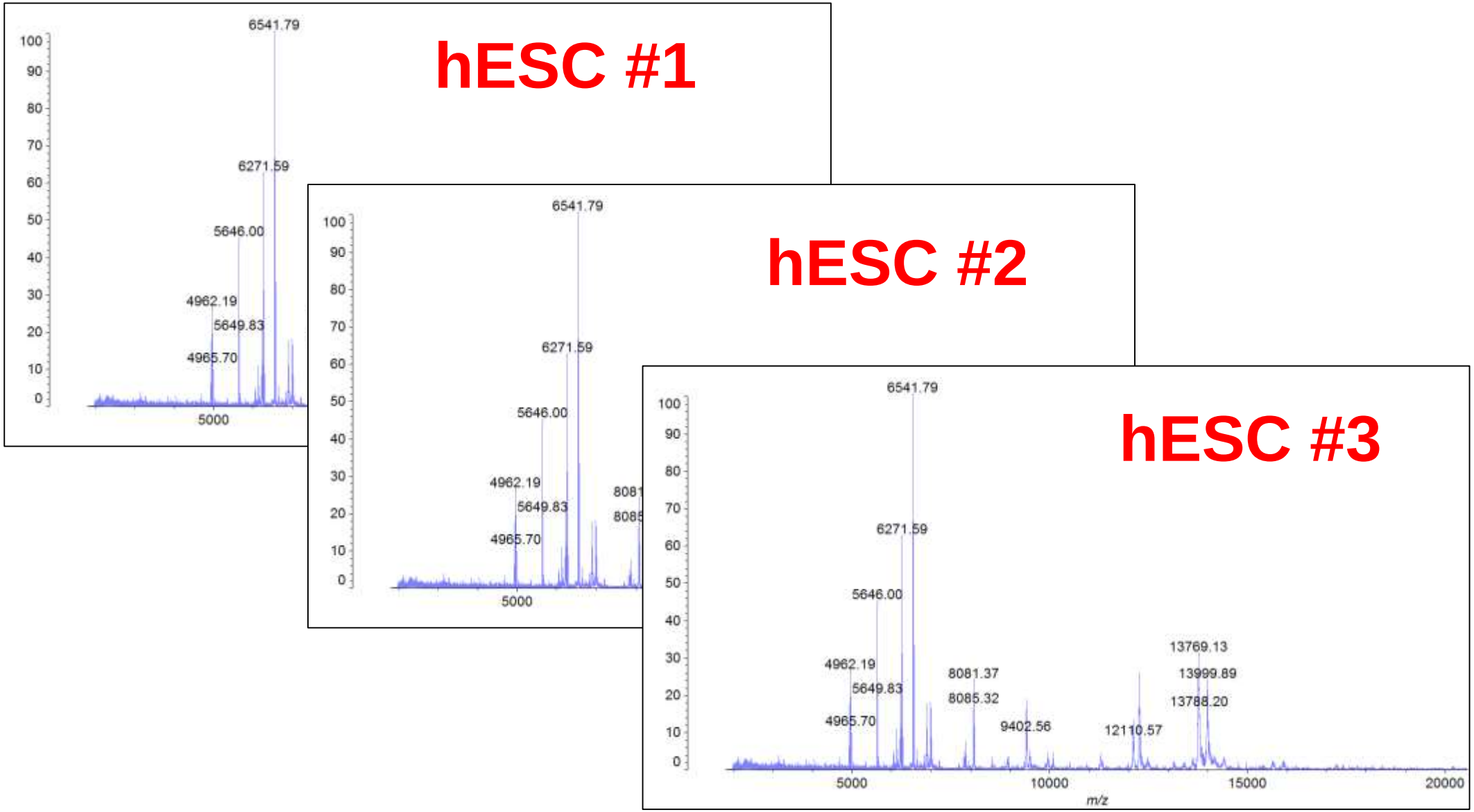
Intact-cell MALDI-TOF MS

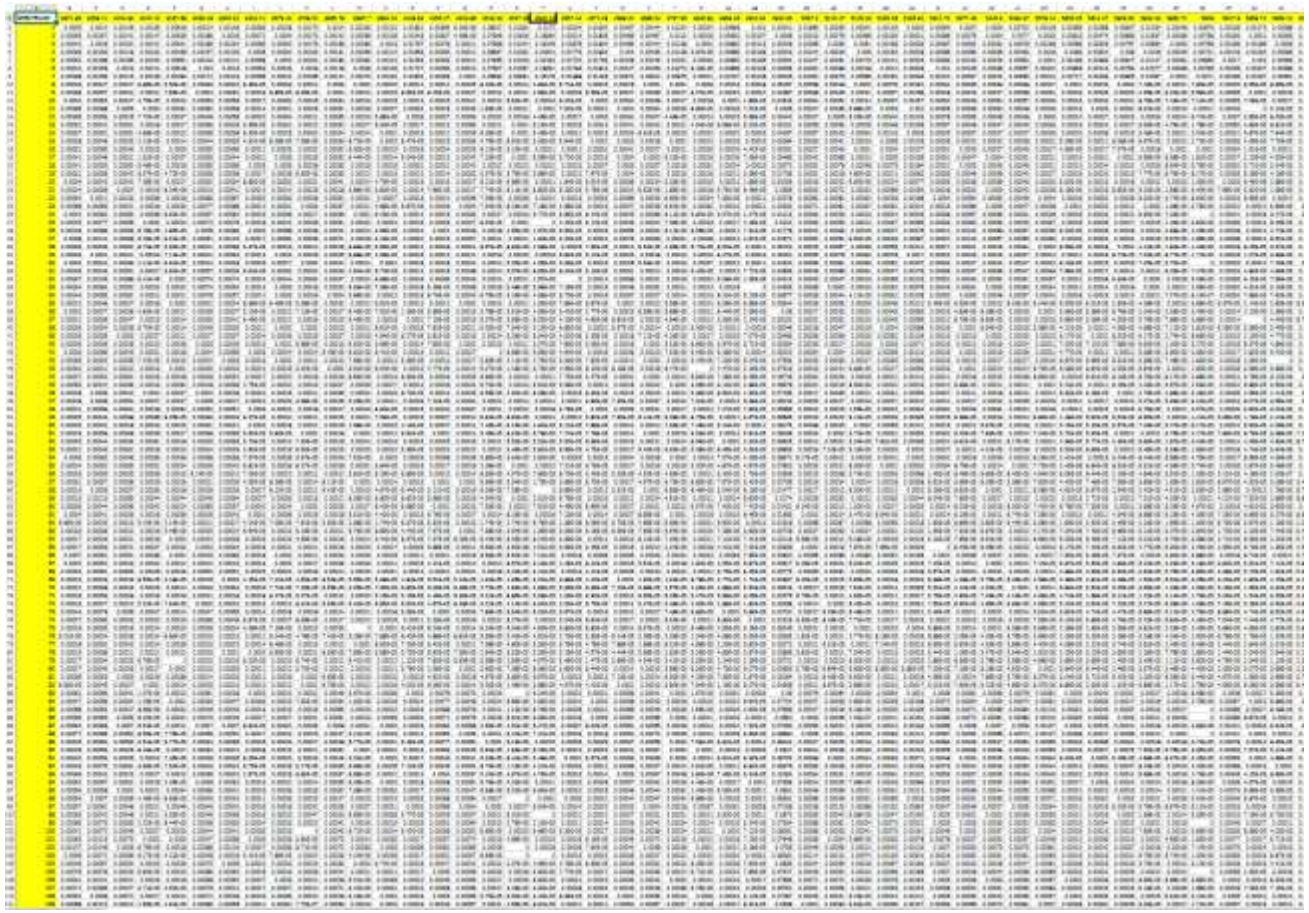


Example of dataset

- “whole spectrum“ approach
- multivariate complex dataset

m/z

Cases



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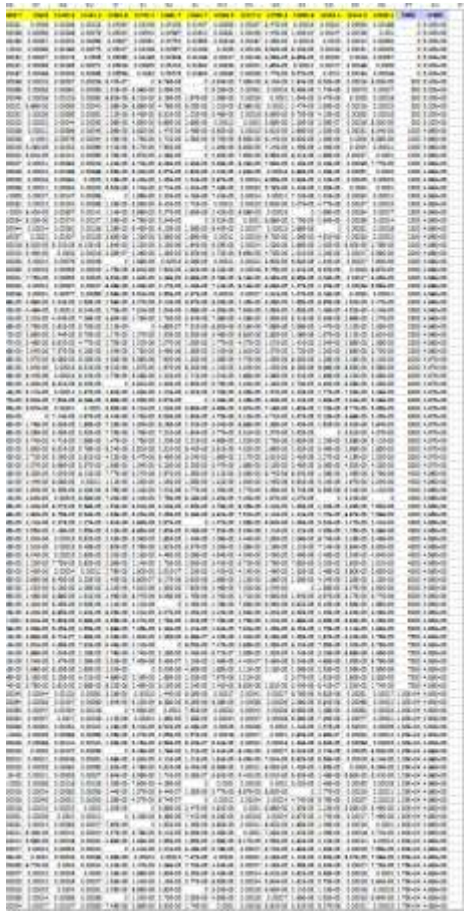

Determination of cell types

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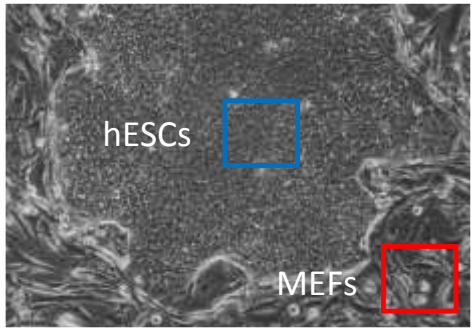
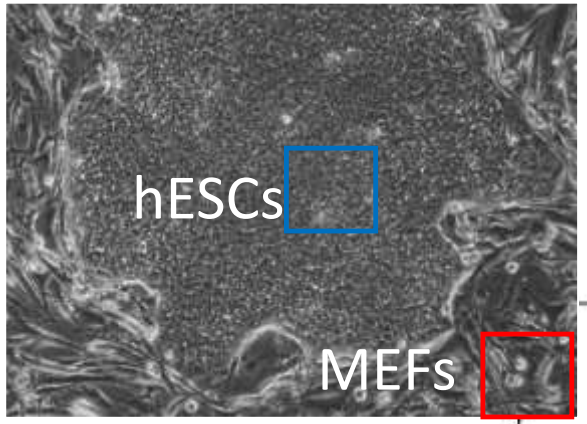
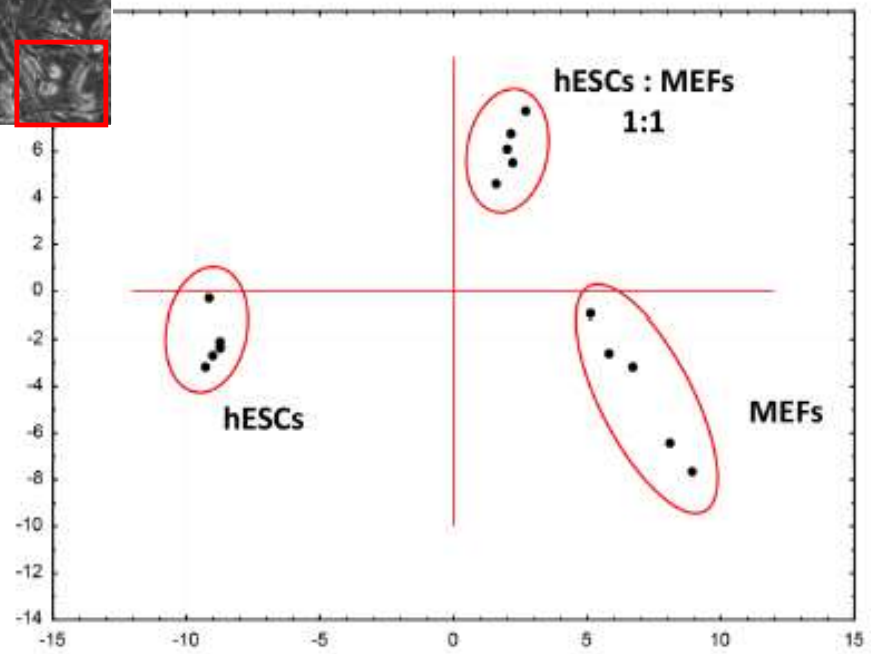
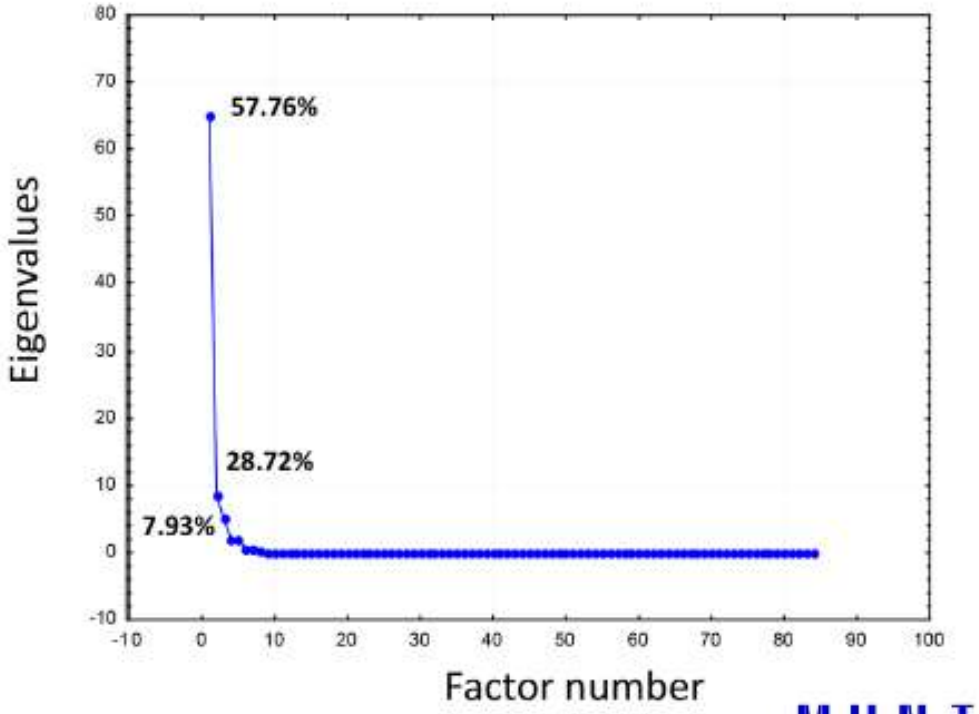
Identification of cell type by MS – proof of principle



Identification of cell type by MS – proof of principle







Prediction of quantification of cell types in mixture

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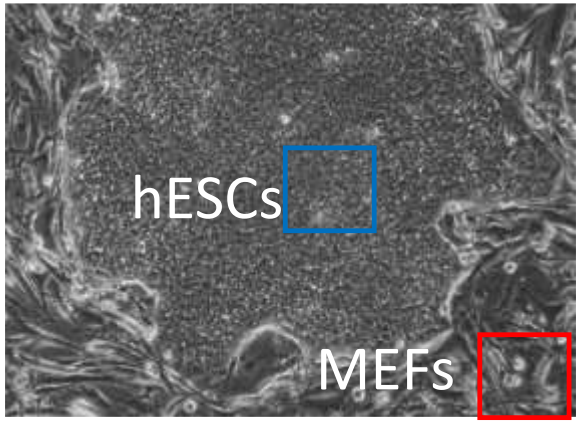
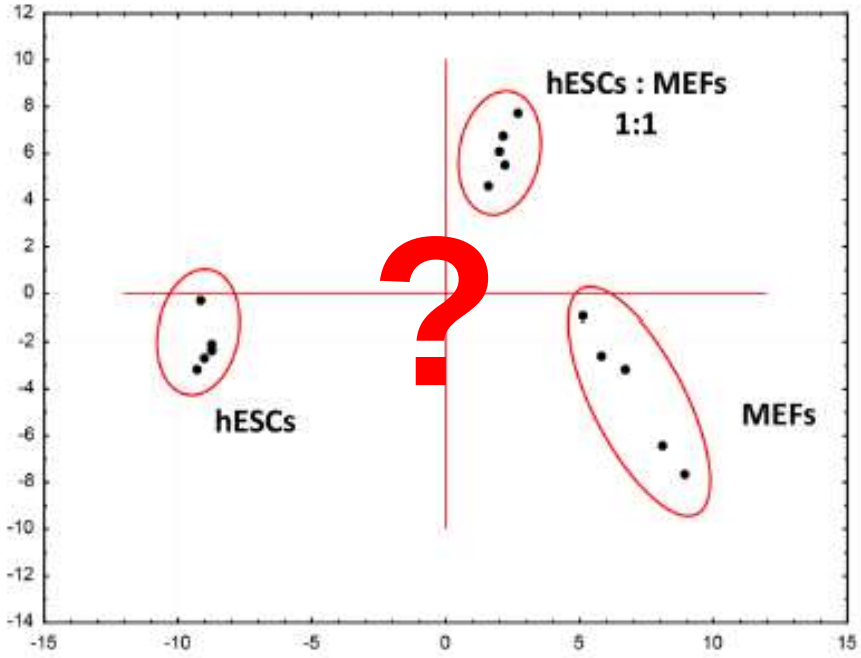
Quantification of cell types in mixture





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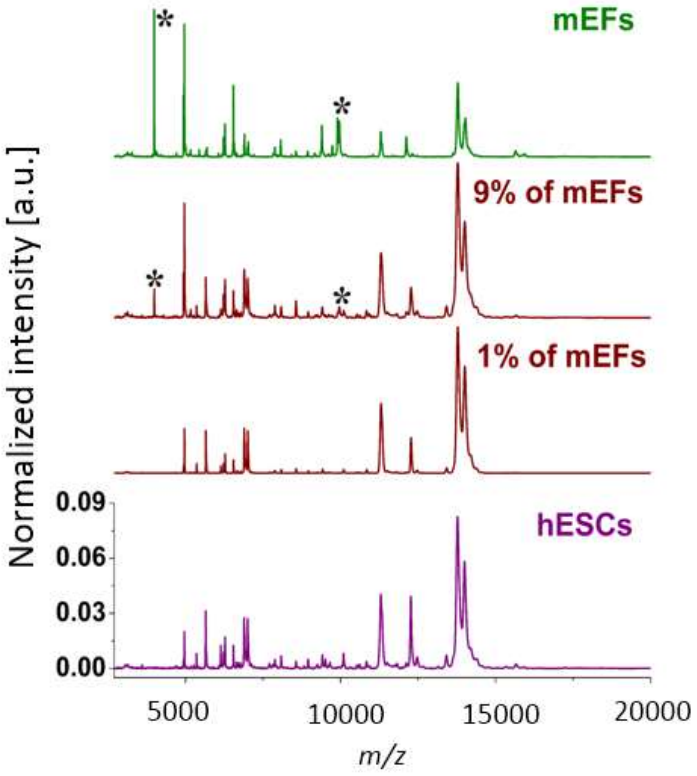
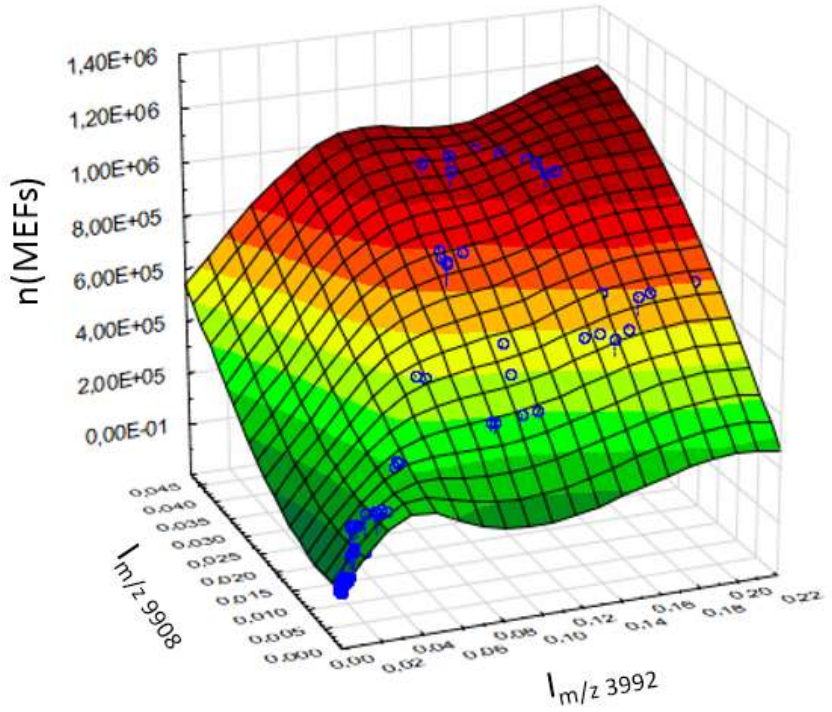
Quantification of cell types in mixture

- “marker peak“ approach **fails**
- problem of **non-linear** relations





Quantification of cell types

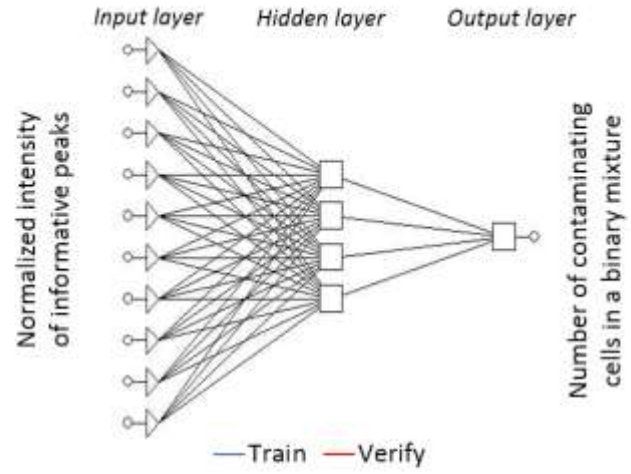
- “Artificial intelligence” approach

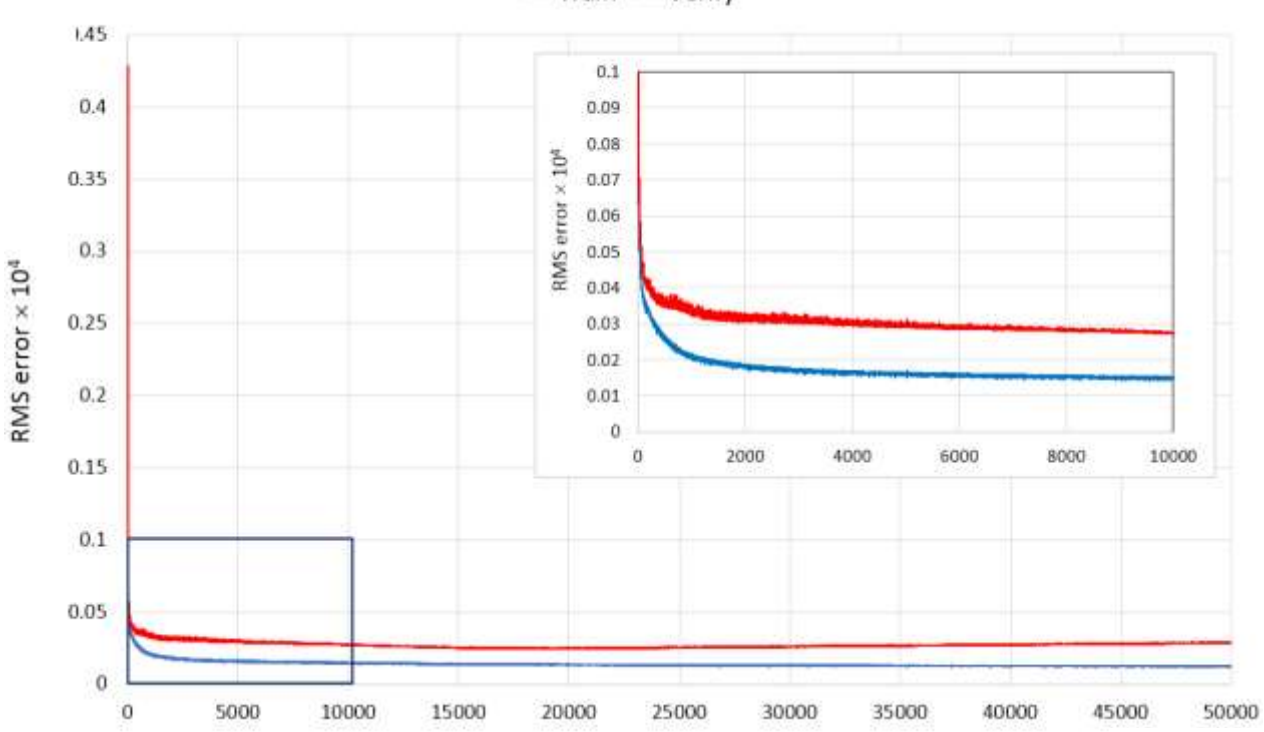
  Multilayer perceptron trained on a database of intensities of selected peaks (m/z) annotated to a particular phenotype

ID	INPUT	OUTPUT
1	Intensities of *n* peaks (n=20-100) of defined *m/z* (2-20 kDa)	Prediction of classification
2		
...		
ID_n		

Training → *Iteration* → **Validation** → **Prediction**

Known input	Unknown input
Assigned to known outputs	Output prediction





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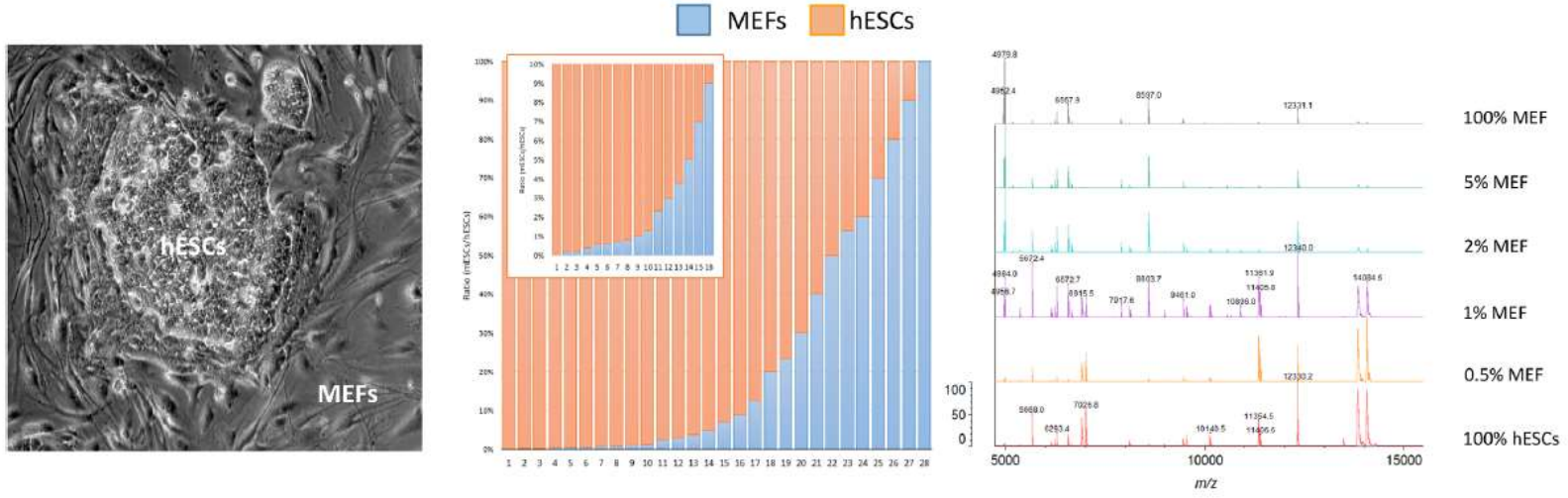
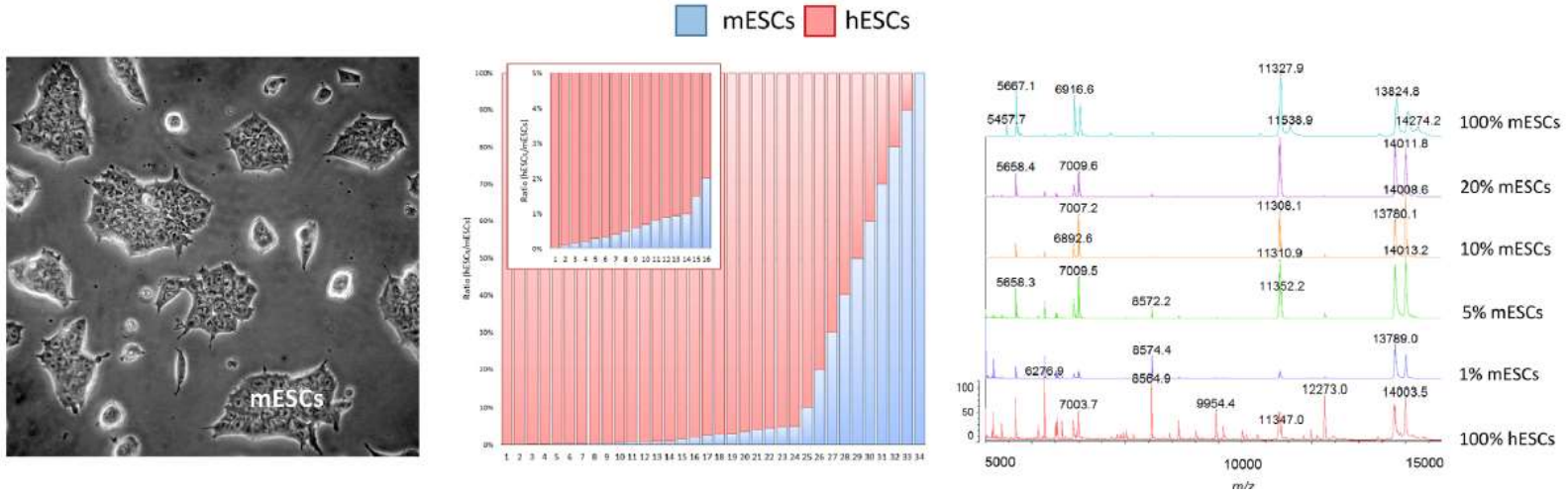
Quantification of cell types

- Multivariate dataset based on binary mixtures of **hESCs : MEFs** or **hESCs: mESCs**

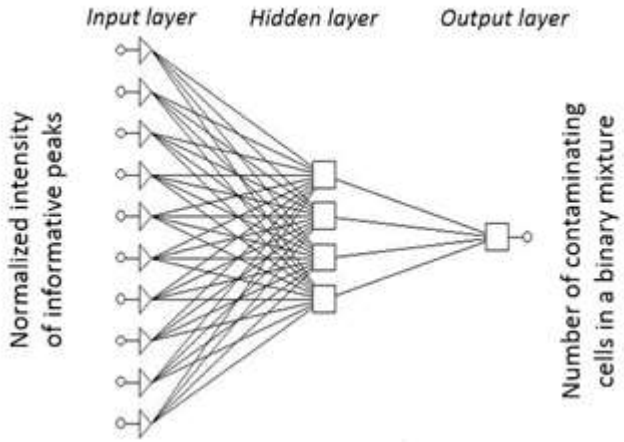


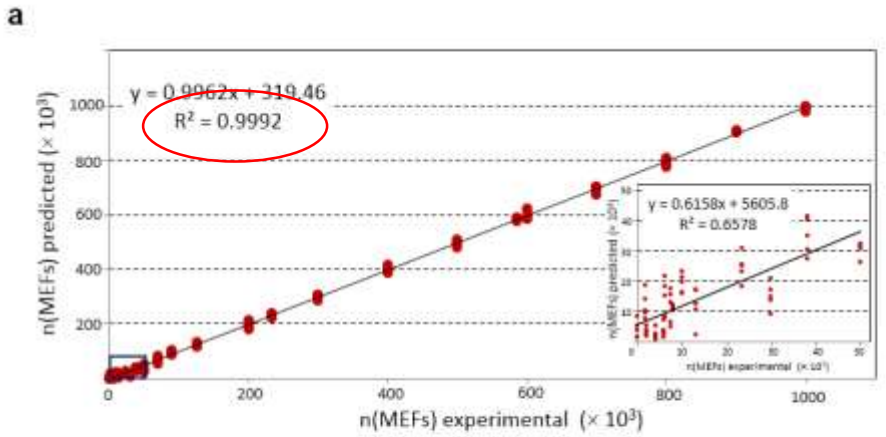


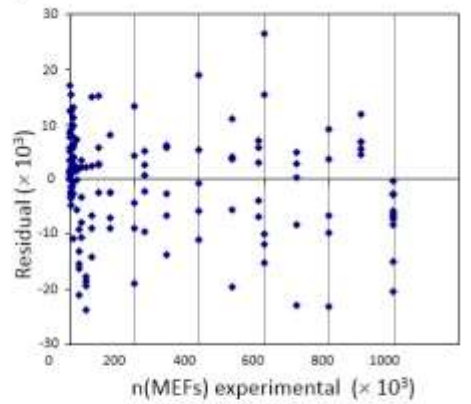
Quantification of cell types

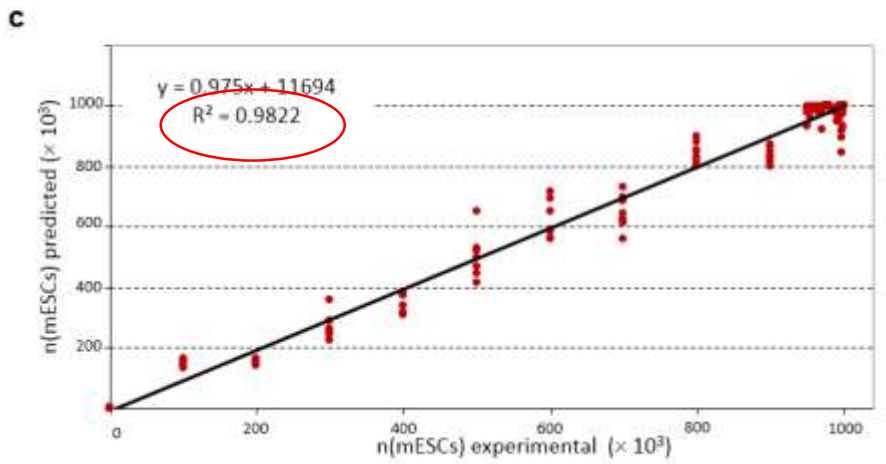
- Prediction by artificial neural network

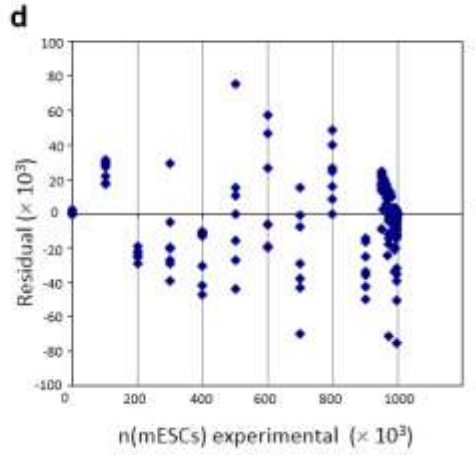
  Correlation of experimental and predicted values

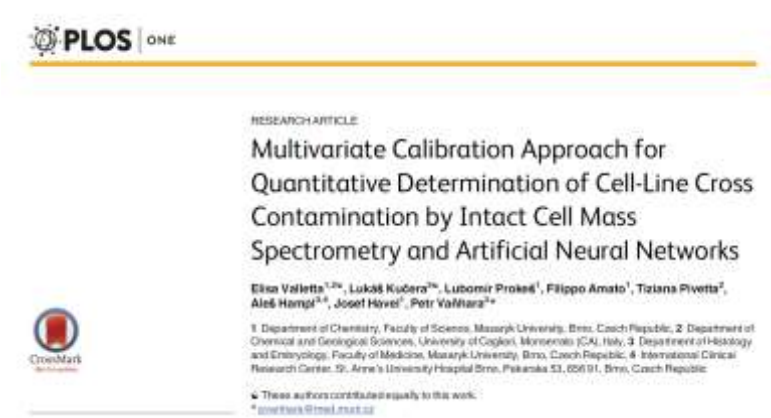











PLOS ONE

RESEARCH ARTICLE

Multivariate Calibration Approach for Quantitative Determination of Cell-Line Cross Contamination by Intact Cell Mass Spectrometry and Artificial Neural Networks

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Real lab-life applications

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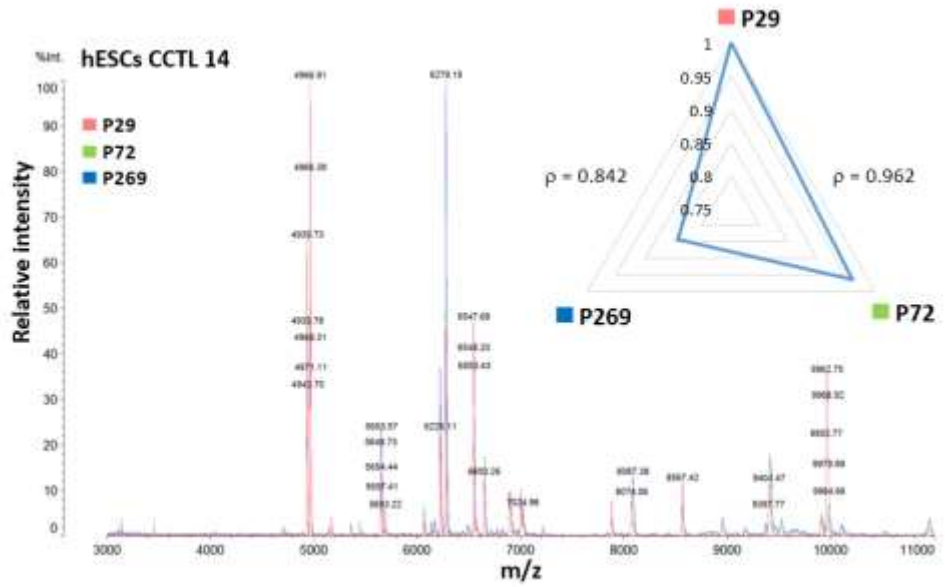
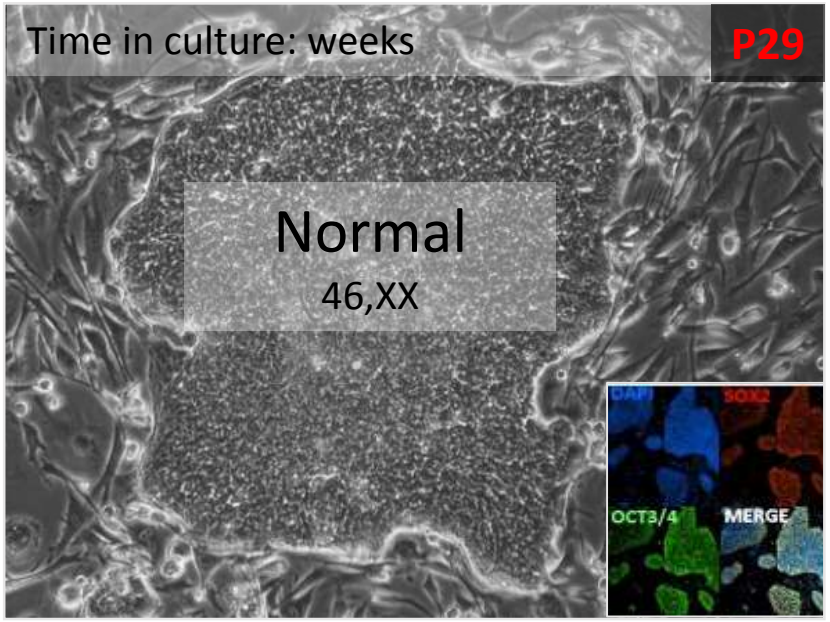
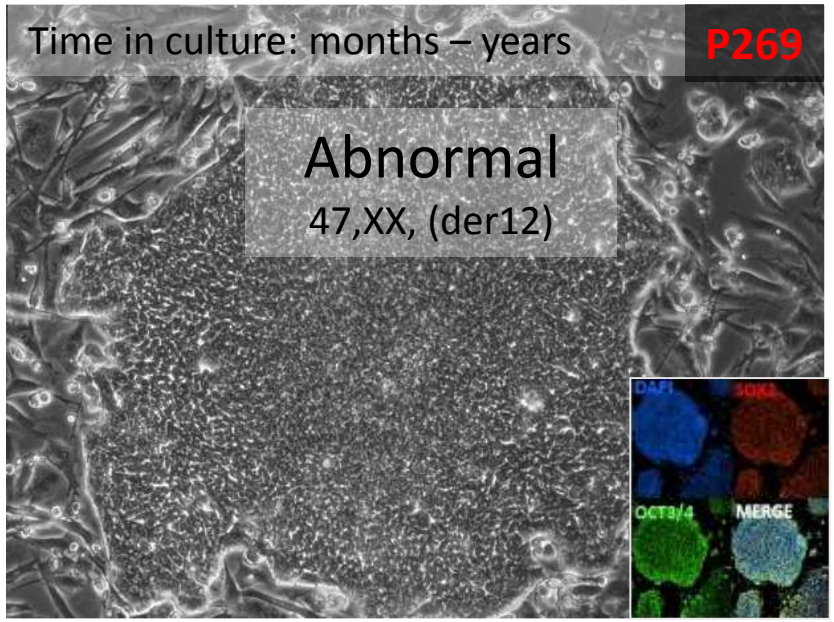
Identification of aberrant hESCs

- identical morphology
- major molecular drivers with negligible fluctuation
- hazardous (cancer prone) phenotype







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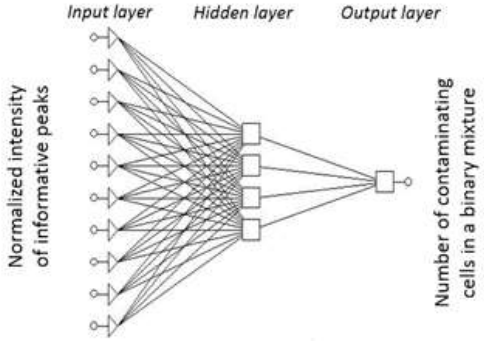
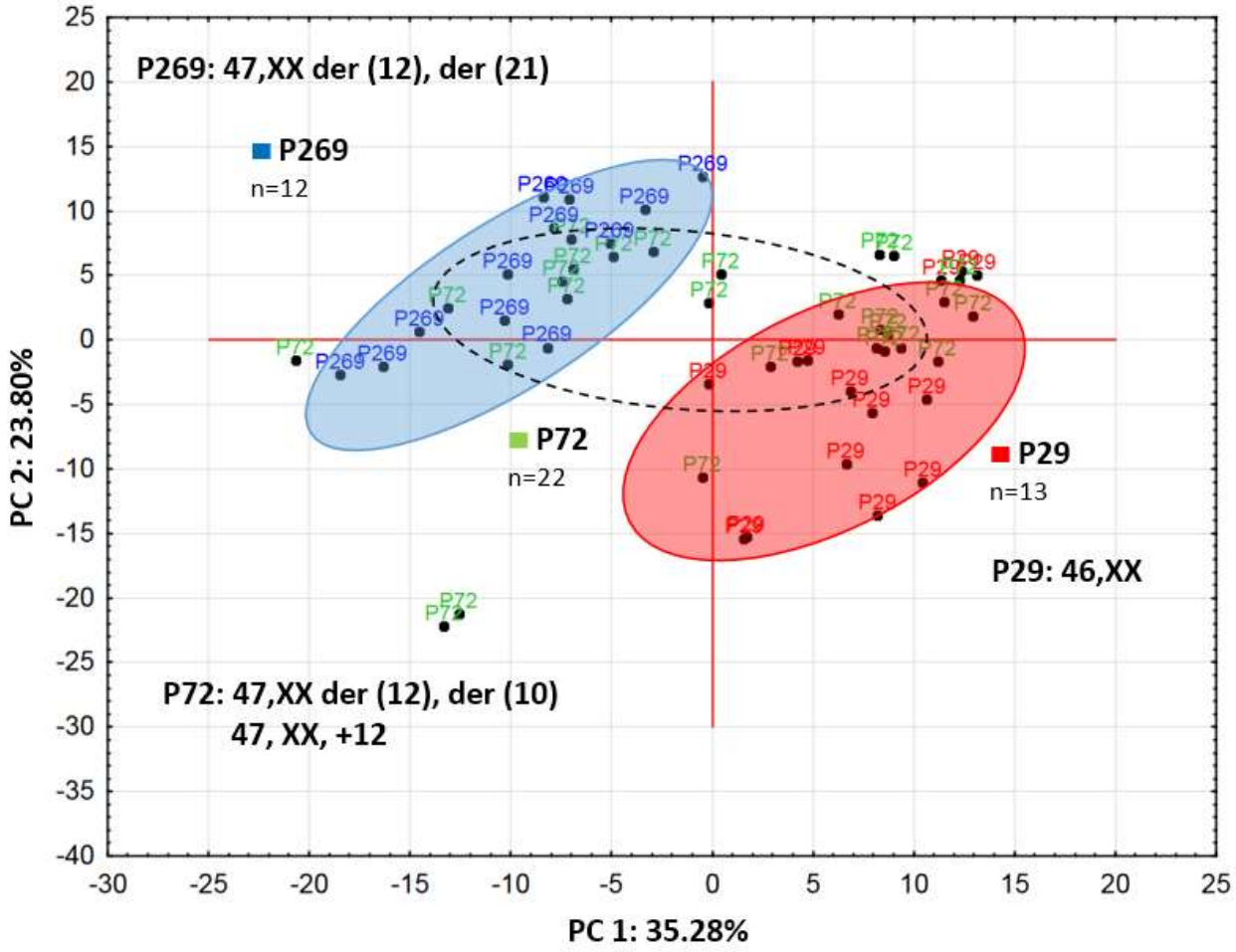
Identification of aberrant hESCs

- based on mass spectrum fingerprints
- correct clustering by PCA

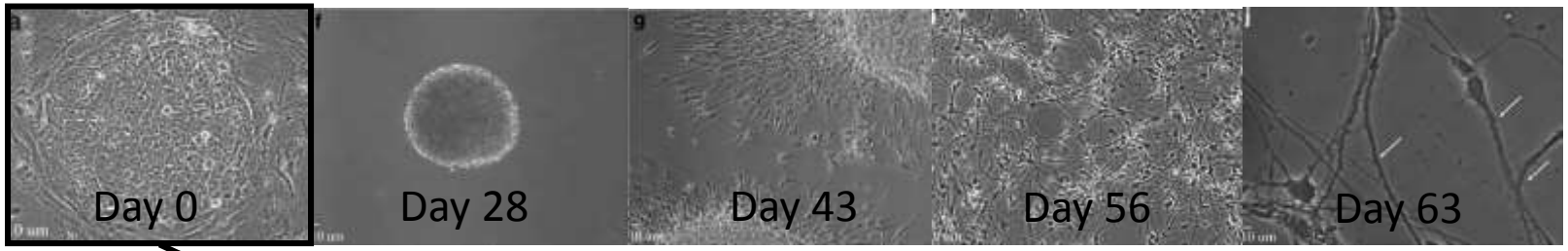


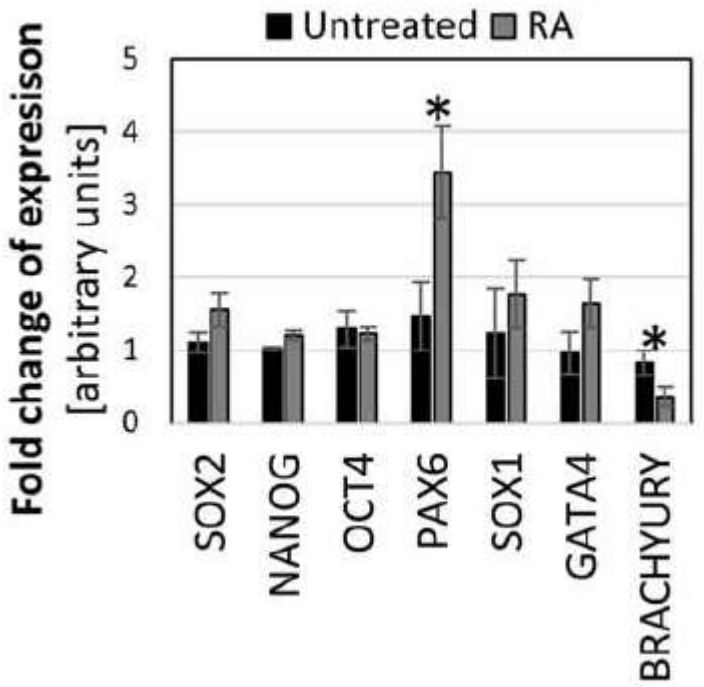


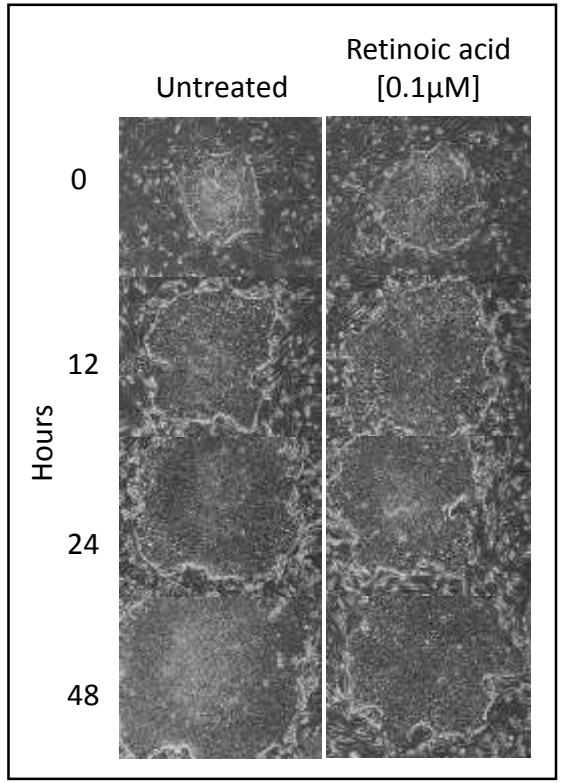
Determination of hESC differentiation

- Modelling retinoic acid-induced neuronal differentiation

Francis and Wei, Cell Death and Disease (2010)







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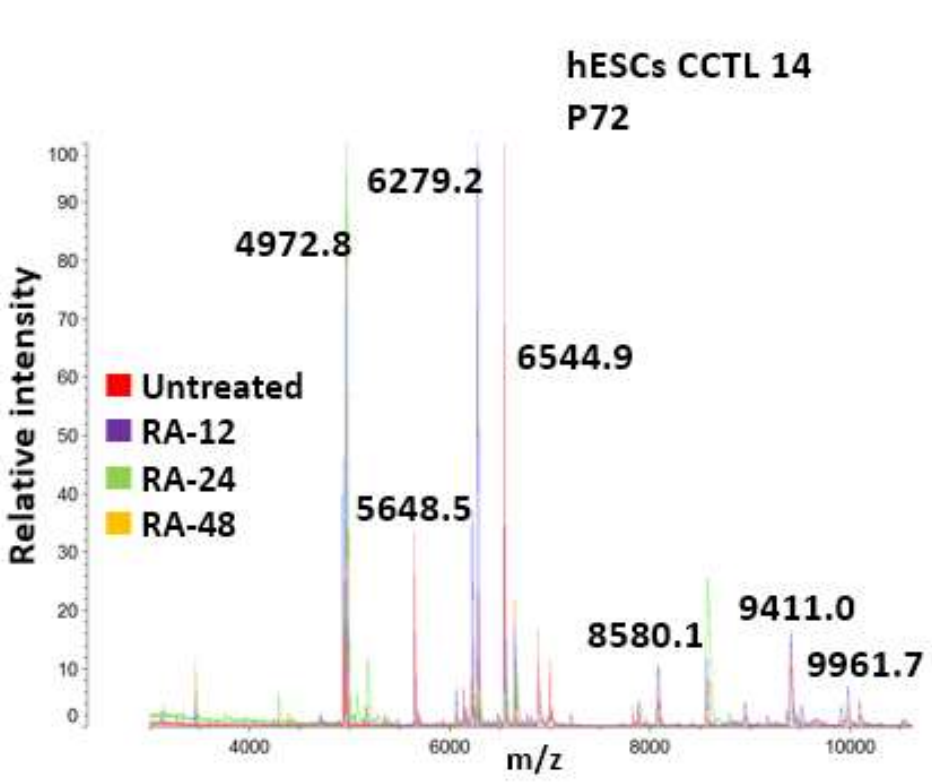
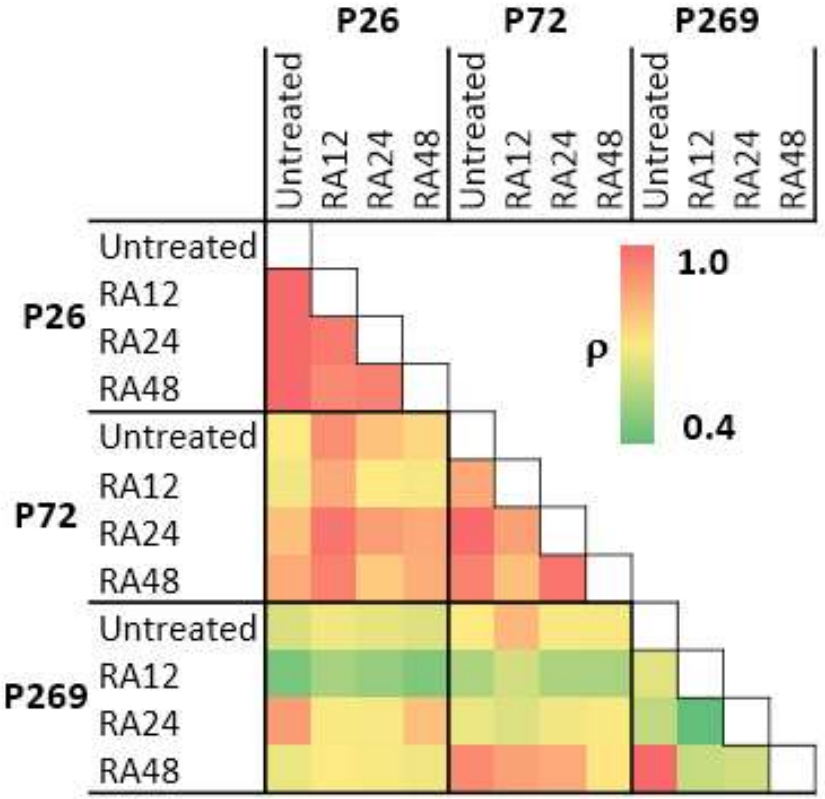
Determination of hESC differentiation

- model of retinoic acid-induced neuronal differentiation





Determination of hESC differentiation

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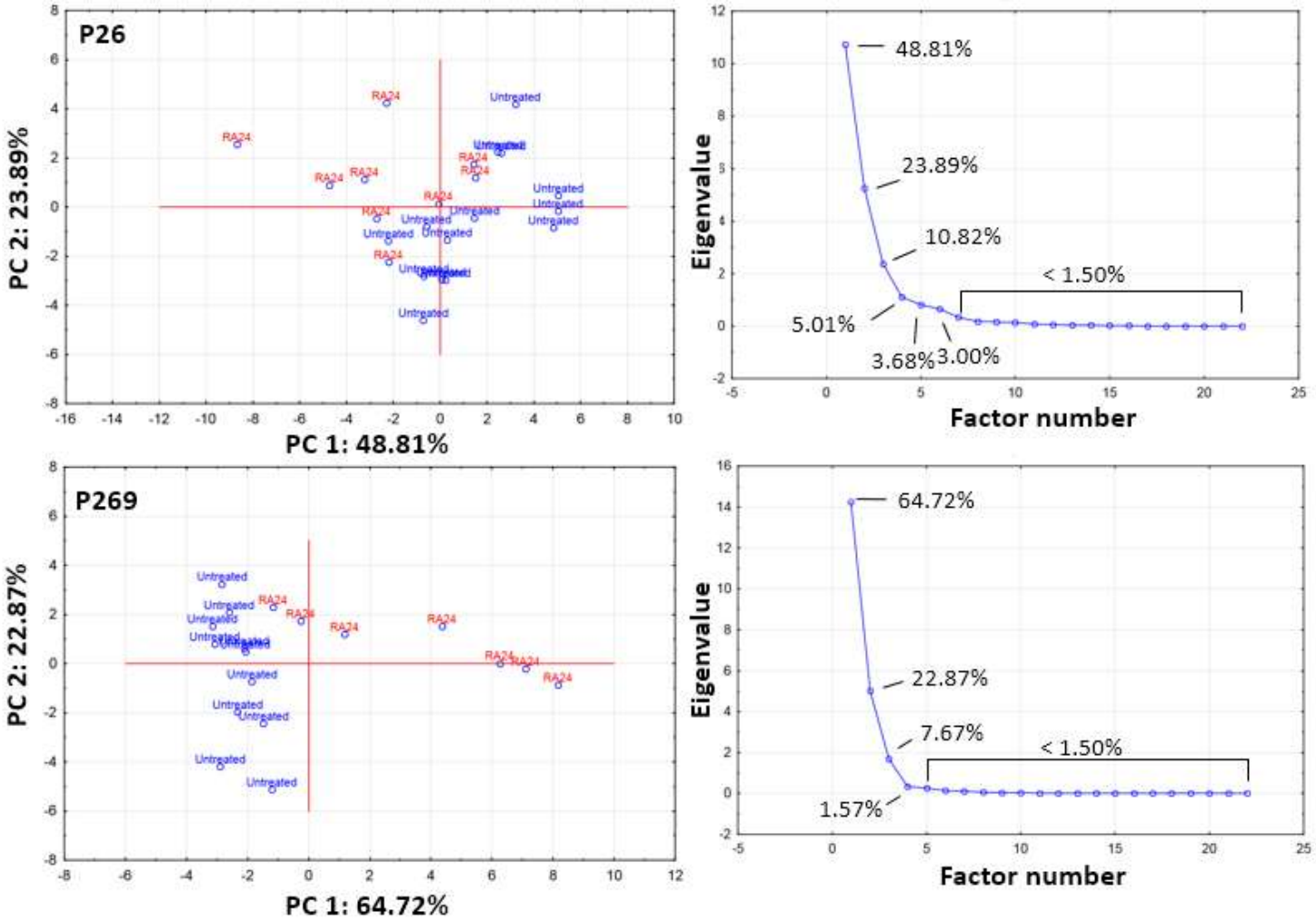
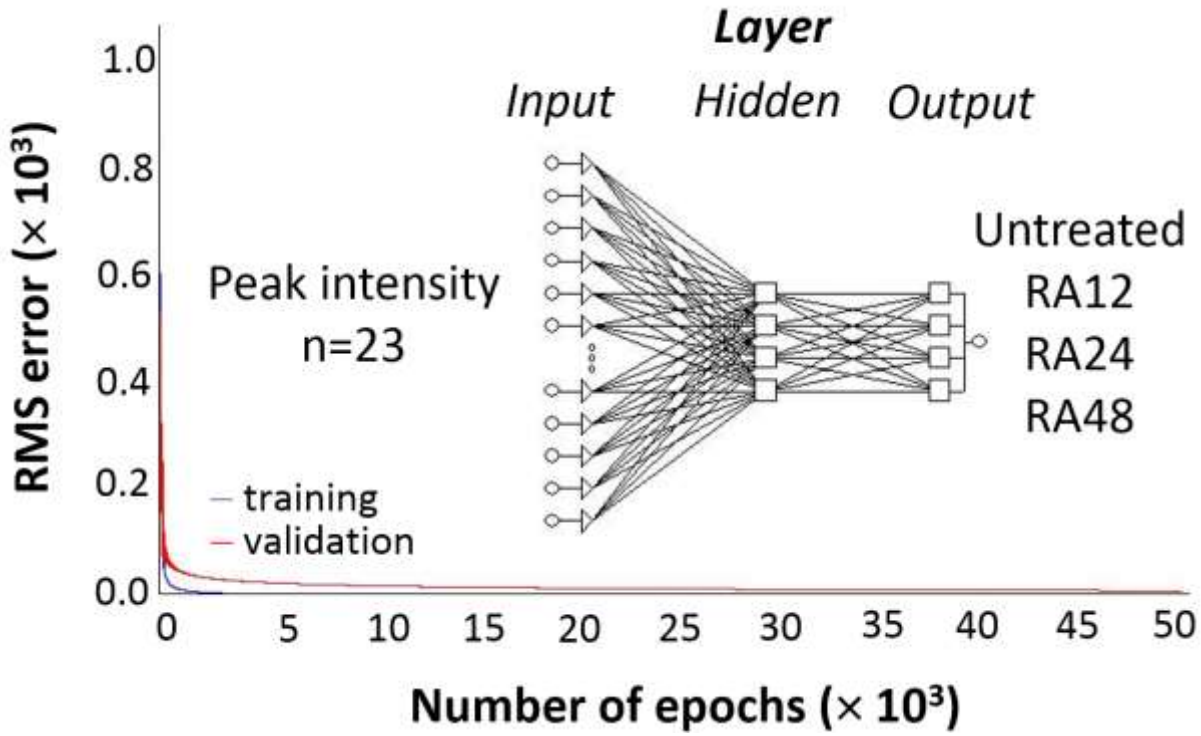


Determination of hESC differentiation

- ANN-based approach
- training using 23 peaks

- successfull determination of “unknown” spectral fingerprint

recognized by stem cells biology community as a promising approach (Stem Cells Portal 2018)



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Intact cell MS for cell biotyping

Strengths

- simple, robust, affordable
- biomarker-free
- intuitive, straight-forward
- high reproducibility within datasets
- discrimination of unapparent but critical alterations in stem/ progenitor cells that are not detected by other techniques
- clinical applications (diagnostics, quality control, clinical grade cell technology)

Weak points

- inter-instrumental variability
- high number of low intensity peaks
- calibration sensitivity
- data processing
- ANN overtraining
- careful translation to routine application or commercialization

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Thank you for attention

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