STUDY OF TUSC3 GENE CHANGES IN OVARIAN CANCER CELLS USING MASS SPECTROMETRY COUPLED WITH BIOSTATISTICAL METHODS

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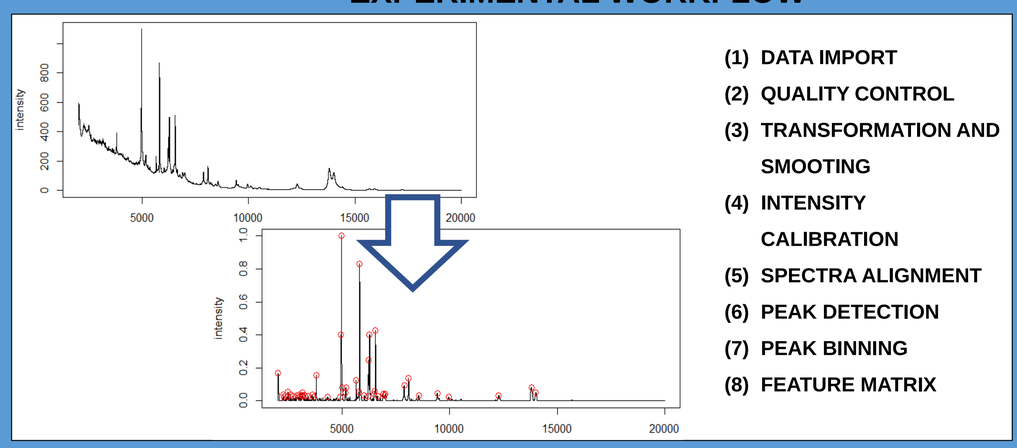
INTRODUCTION

Intact cell mass spectrometry emerged as a promising tool for biotyping and monitoring of cell cultures of various origin, including stem or cancer cells. In our previous work, we demonstrated the efficacy of the method in revealing metabolic or phenotypic changes occurring in cultured cells [1]. In this work, we were curious whether we can distinguish cells differing in expression of a single gene. As a model, we choose Tumor Suppressor Candidate 3 (TUSC3) gene. TUSC3 is a subunit of enzymatic complex (oligosaccharyltransferase) responsible for final steps of N-glycosylation in endoplasmic reticulum. When TUSC3 is silenced in ovarian cancer, it promotes aggressiveness of the disease and limits survival of patients. In cultured cells, it induces profound phenotypical changes, most probably due to alterations in the glycoproteome. Previously, we have established model cell lines with silenced TUSC3 and described them thoroughly [2-4].

Here we demonstrated that intact cell mass spectrometry can clearly discriminate cells differing in expression of a single gene.

EXPERIMENTAL WORKFLOW

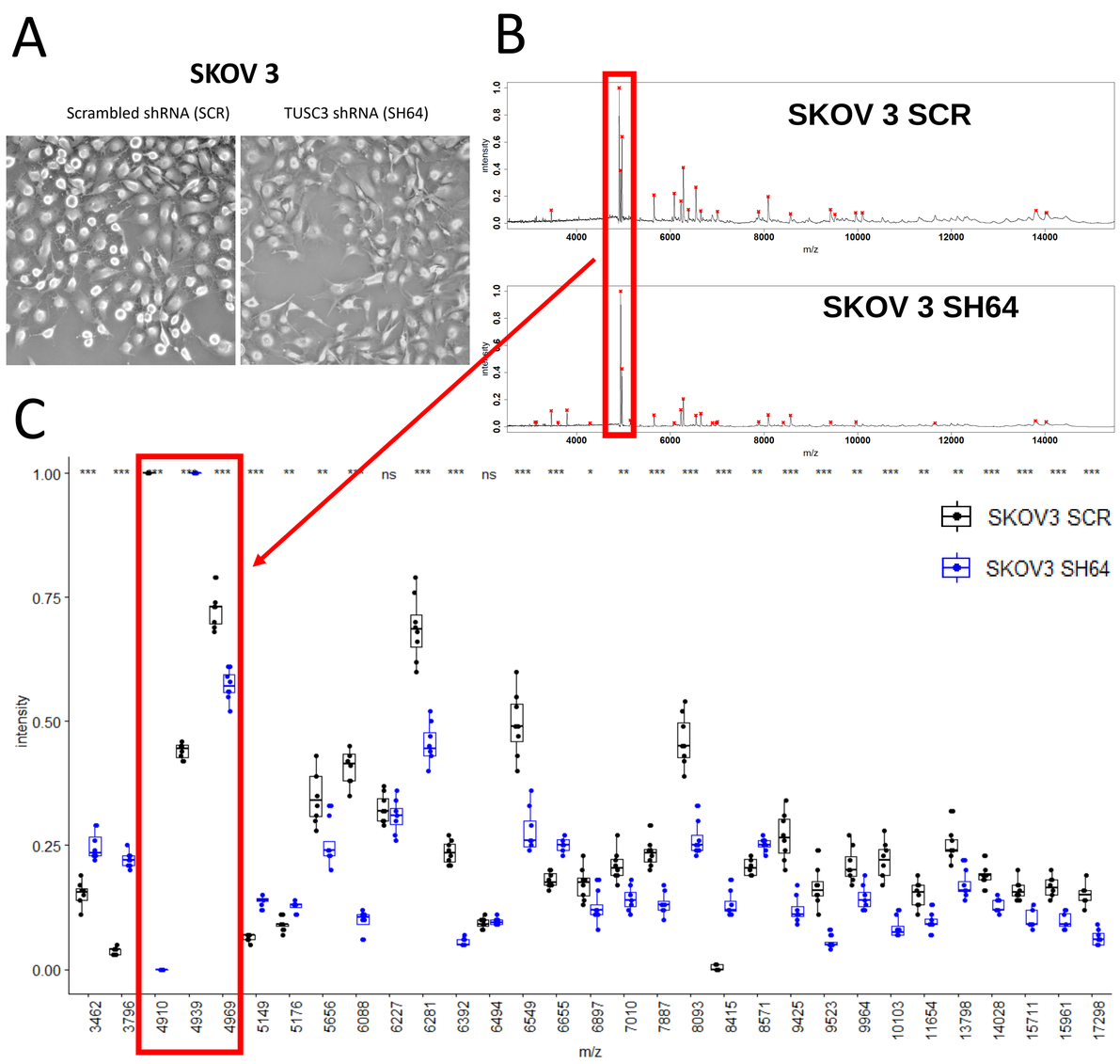


Cells were cultured under standard conditions, manually harvested, washed in MS-compatible buffers, mixed with acidified matrix and directly spotted on MALDI target. Intact cell mass spectra were pre-precessed using R Studio or eMSTAT solution software.

For experimental details see [1] or :

RESULTS AND DISSCUSION

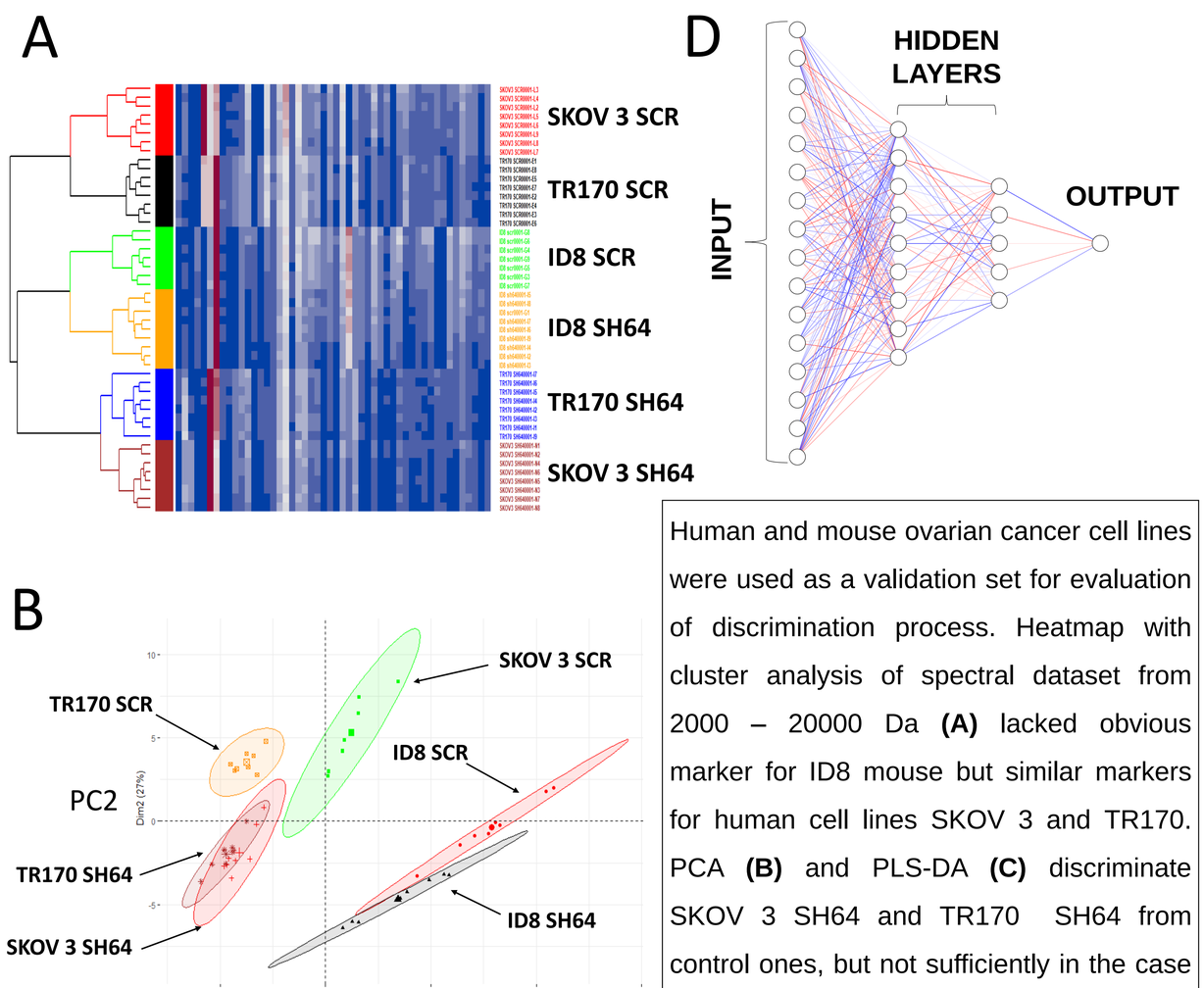
PROOF OF PRINCIPLE



Representation of morphology of cultured human ovarian cancer cell line SKOV 3 with silenced TUSC3 (SH64) and the respective scrambled control (SCR) **(A)**. Cells were manually harvested, washed and processed by Intact cell MALDI-MS **(B)**. The spectral fingerprints show possible marker peaks, when the most obvious are peaks at m/z 4910, 4939, and 4969 respectively **(C)**. PCA of spectral datasets correctly clustered cells with and without expression of TUSC3 gene. Each point in the PCA plot represents a unique biological sample **(D)**.

In summary, spectral fingerprints discriminate SKOV3 SH64 cells from the control ones SKOV3 SCR.

VALIDATION IN PANEL OF CELL LINES



Human and mouse ovarian cancer cell lines were used as a validation set for evaluation of discrimination process. Heatmap with cluster analysis of spectral dataset from 2000 – 20000 Da **(A)** lacked obvious marker for ID8 mouse but similar markers for human cell lines SKOV 3 and TR170. PCA **(B)** and PLS-DA **(C)** discriminate SKOV 3 SH64 and TR170 SH64 from control ones, but not sufficiently in the case of ID8. Dataset of PC1 – PC*n* was used as input for ANN **(D)**, trained, and used for classification. The ANN correctly classified the unknown mass spectrum to appropriate sample group

In summary using coordinates from PCA as input for non-linear, self learning approaches ANN leads to improved discrimination selected cells to individual classes.

CONCLUSIONS

Intact cell MALDI TOF MS can discriminate cells differing in the expression of a single gene.

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