Proteomic fingerprint of rearrangement of the lung function in a breast cancer lung metastases – in a search for the molecules for detection of metastases and potential therapeutic targets

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Introduction

Breast cancer is one of the most common cancers. It is characterized by a strong ability to metastasize, specifically to lungs (the incidence of metastasis is above 60%), and consequently results in high mortality.

Objective

The study aimed at the proteomic characterization of the changes in the function of lungs in relation to metastases development in the experimental model of tumour metastasis in murine 4T1 mammary adenocarcinoma.

Results

Histological analysis confirmed the presence of the metastases in the 4th week after the tumour cell inoculation. The label-free proteomic analysis revealed 1585 and 1561 proteins in the control group and metastatic lungs respectively. The 168 differentially expressed proteins were involved in multiple biological processes related to *i.a.*: gene expression, cell differentiation, cellular response to stimulus, cellular developmental process, organelle and cellular component organization, localization, response to stress, multiple metabolic processes *e.g.*: organic substance metabolic process, primary metabolic process, nitrogen compound metabolic process. Most of the differentially expressed proteins were involved in binding of different molecules. The results for complementary techniques used in the study greatly overlapped, with additional information provided by label-free comparative analysis. The prominent changes were reported for proteins involved in *i.a.* immunological response *e.g.*: protein S100-A9, protein S100-A8, cathelicidin antimicrobial peptide, integrin alpha M, neutrophilic granule protein, myeloperoxidase, neutrophil cytosol factor 2, retinoic acid-induced protein 3, myosin-11, cysteine-rich protein 2, dipeptidyl peptidase 4, decorin, mimecan.

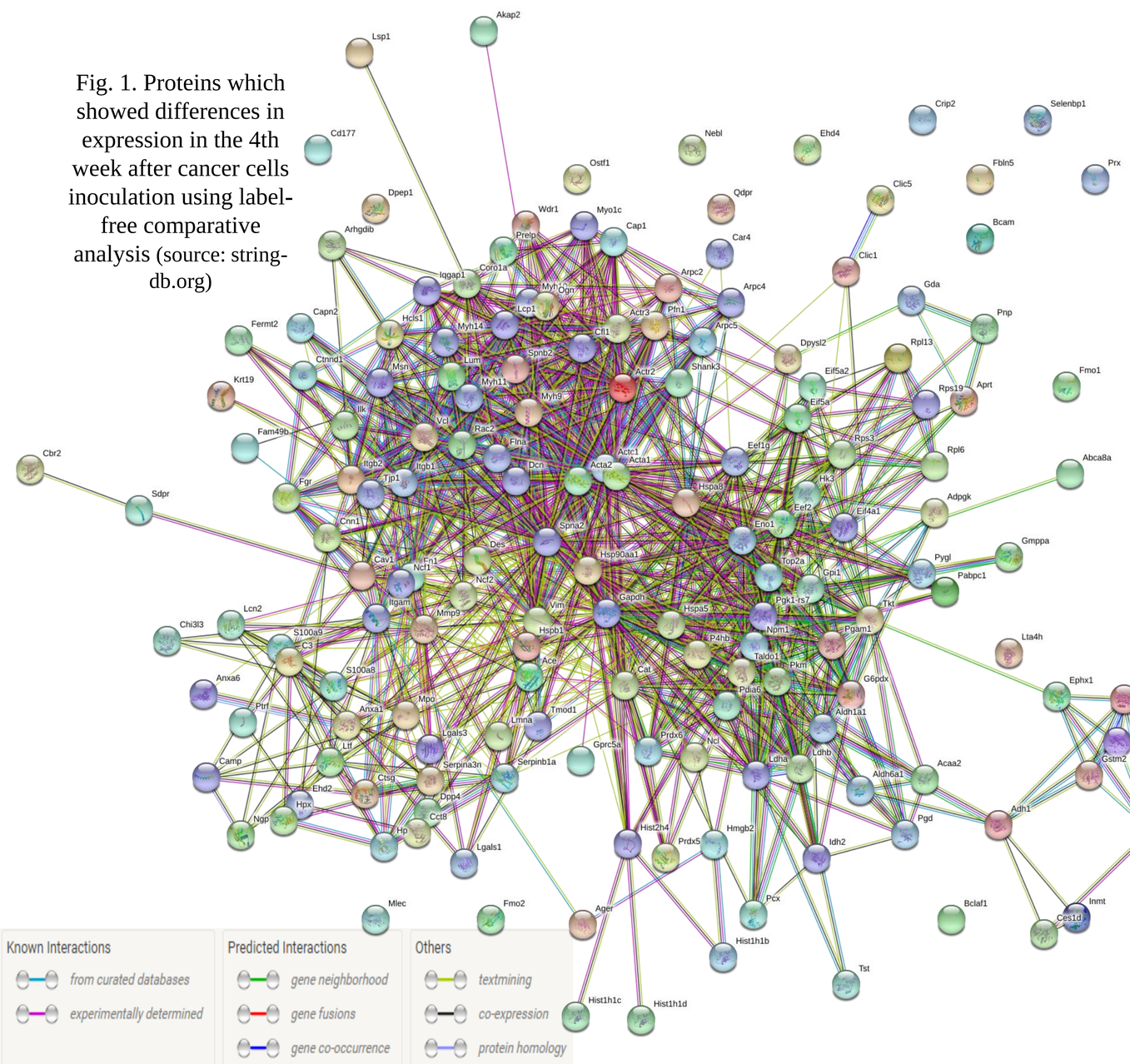


Fig. 1. Proteins which showed differences in expression in the 4th week after cancer cells inoculation using label-free comparative analysis (source: string-db.org)

Materials and methods

The 4T1 tumor cells were orthotopically inoculated into the mammary fat pad of the BALB/c female mice. Analysis was performed in lungs 4 weeks after cancer cell transplantation. 2D-DIGE as well as label-free comparative proteomics with the aid of nanoLC-MS/MS technique were applied.

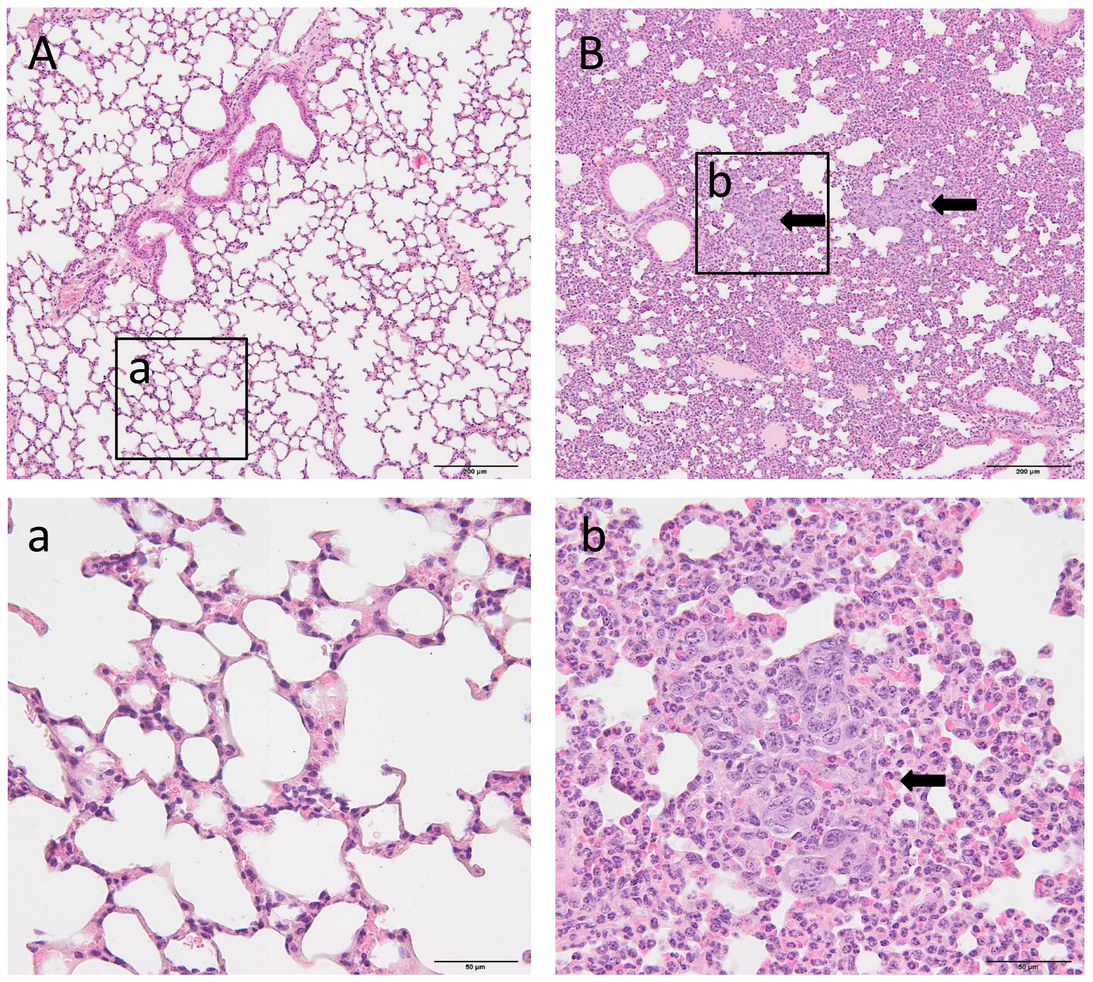
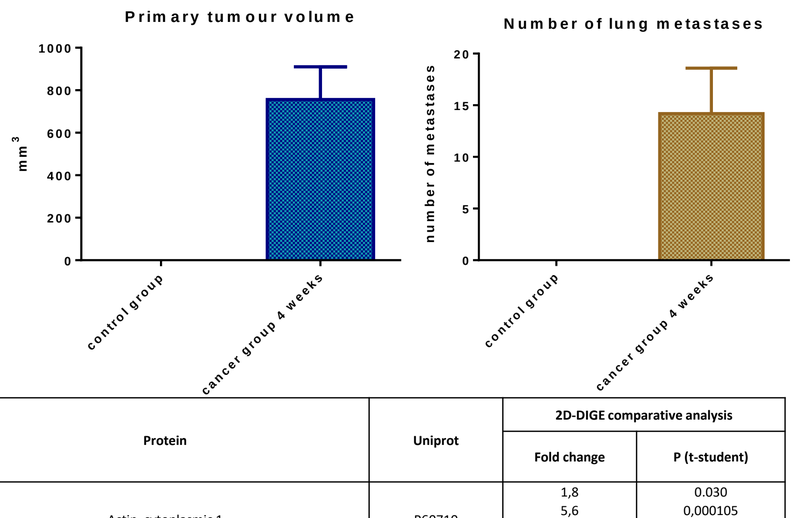


Fig. 2. Histology of the lung in the control group (A-100x, a-400x, and 4th week after inoculation of 4T1 tumour cells (B-100x, b-400x)

Fig. 3. Primary tumor volume and numer of lung metastases in the control group and the 4th week after inoculation of 4T1 tumour cells



Label-free comparative analysis		Protein	Uniprot	2D-DIGE comparative analysis	
q-value	Ratio			Fold change	P (t-student)
0,21450	1,09	Actin, cytoplasmic 1	P60710	1,8 5,6 2,6 2,0	0.030 0,000105 0,004 0,00054
		Ubiquitin-conjugating enzyme E2 K	P61087	1,7	0.0006143
0.00007	1,14	Vimentin	P20152	2,0	0.015
0.00004	2,37	Rho GDP-dissociation inhibitor 2	Q61599	1,6 1,6	0.039 0,015
0.98881	1,13	Ferritin heavy chain	P09528	2,1	0.012
0.00004	3,60	Neutrophilic granule protein	O08692	1,8 1,9 1,7 2,4	0.036 0.014 0,018 0,01
0.00004	0,81	Caveolae-associated protein 1	O54724	2,0	0.02
		Tubulin alpha-1C chain	P68373	2,9	0.0006925
0.00019	5,15	Protein S100-A9	P31725	2,0 6,6	0.011 0.002
1.00000	1,05	Coactosin-like protein	Q9CQI6	1,6	0.033
0.00004	2,12	Leukocyte elastase inhibitor A	Q9D154	2,0	0,001
0.53187	0,92	Tropomyosin alpha-4 chain	Q6IRU2	1,8 (-) 1,8 (-)	0.004 0.042
0.82066	1,15	Serine protease inhibitor A3K	P07759	1,6 (-)	0.016
1.00000	0,93	Serum albumin	P07724	1,8	0.008
0.98060	0,91	Tropomyosin alpha-1 chain	P58771	1,5	0.031
0.52878	0,96	Synaptic vesicle membrane protein VAT-1 homolog	Q62465	1,7	0.014
0.79505	1,03	Sorting nexin-6	Q6P8X1	1,7	0.008

Table 1. Proteins which showed differences in expression in the 4th week after cancer cell inoculation using 2D-DIGE and results obtained using label-free comparative analysis for the presented proteins (source: string-db.org)

Conclusions

The proteomic profiling enabled to reveal the pattern of changes in metastatically affected lungs. Differentially expressed proteins may be considered in further analysis as potential therapeutic targets as well as the fingerprints for discerning between healthy and metastatic tissue.

References: Nicolini et al. (2017) Seminars in Cancer Biology S1044-579X(17)30052-30054; Brinton et al. (2012) Cancer Genomics and Proteomics 9(6):345-355; Lobo et al. (2017) Frontiers in Oncology 7:14; Yan et al. (2014) Science China Chemistry 57(5):718-722; Sigloch et al. (2017) Journal of Cancer 8(19):4065-4074.

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