Premetastatic rearrangement of lungs function – proteomic characterization of metastatic niche formation

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Introduction

Metastases are the major cause of high mortality in patients with cancer [1]. Lung metastatic niche facilitate the process of cancer cells survival in a foreign microenvironment and enables their protection against immune defense [2,3].

Objective

The study aimed at the proteomic profiling of the lung tissue in order to characterize the mechanisms underlying the pre-metastatic rearrangement of lung function, thus the pre-metastatic niche formation in the experimental model of tumor metastasis in murine 4T1 mammary adenocarcinoma.

Results

The 4T1 tumor cells were orthotopically inoculated into the mammary fat pad of the BALB/c female mice. Analysis was performed in lungs 1 and 2 weeks after cancer cells transplantation. 2D-DIGE was applied for the comparative analysis of protein expression patterns with nanoLC-MS/MS technique for identification of differentially expressed proteins.
The investigated weeks represented the metastatic niche formation period as the metastases appeared in the lungs after the 2nd week after cancer cells inoculation. Slight but statistically significant changes were noted in structure, binding, transporter/receptor, catalytic and antioxidant activity. In context of further selection of early cancer-related biomarkers the most interesting were *i.a.*: endoplasmin, serine protease inhibitor a3k, selenium binding protein,14-3-3 protein zeta/delta, EF-hand domain-containing protein D2, Rho GDP-dissociation inhibitor 2 and calumenin.

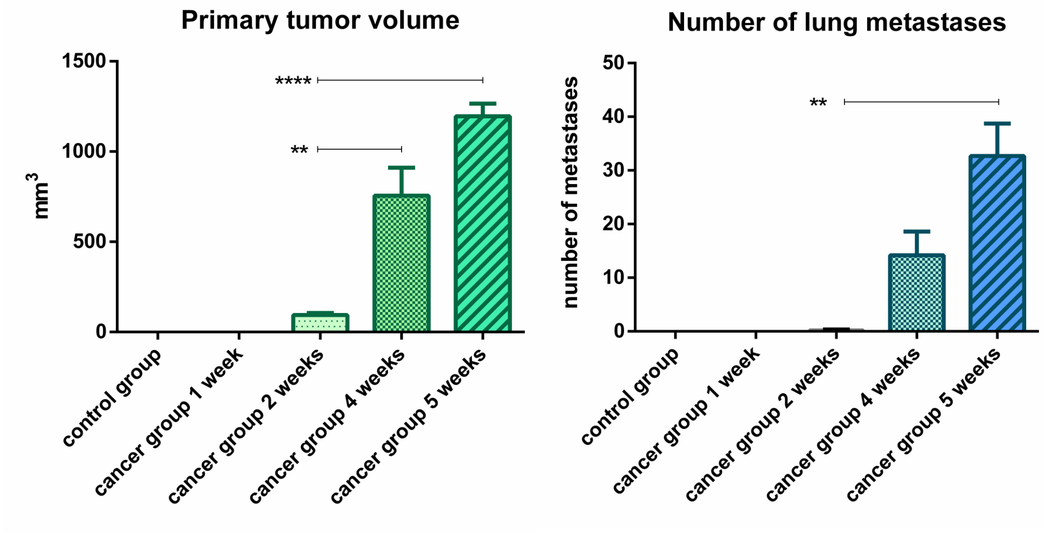


Fig. 1. Primary tumor volume and numer of lung metastases in subsequent weeks after inoculation of 4T1 tumour cells

Conclusions

This approach enabled the characterization of the molecular changes and to analyze the adaptation mechanisms of the system, which gives great opportunities to understand the metastasis progression and identify potential biomarkers characteristic for the early stages of cancer metastasis, thus identification of the potential targets for therapies.

References:
[1] Weigelt et al., 2005. Breast cancer metastasis: markers and models. Nat Rev Cancer 5:591-602.
[2] Zheng et al., 2013. Analysis of differentially expressed proteins involved in metastatic niche of lung. Thoracic Cancer 4:385-394.
[3] Maru Y, 2013. The lung metastatic niche. J Mol Med (Berl) 93(11):1185-1192.

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 1 and 2 weeks after cancer cells transplantation. 2D-DIGE was applied for the comparative analysis of protein expression patterns with nanoLC-MS/MS technique for identification of differentially expressed proteins.

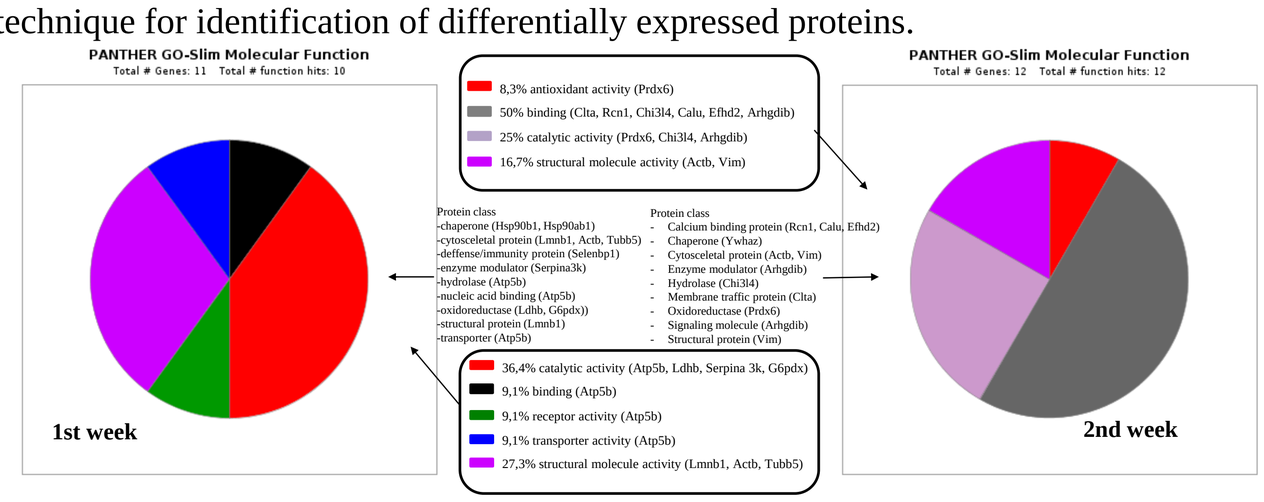


Fig. 2. Classification of differentially expressed proteins according to molecular function and protein class (source: pantherdb.org)

Protein	Gene
Actin, cytoplasmic 1	Actb ↓
Endoplasmin	Hsp90b1↑
Heat shock protein HSP 90-beta	Hsp90ab1 ↑
Stress-70 protein, mitochondrial	Hspa9 ↓
Lamin-B1	Lmnb1 ↓
Serine protease inhibitor A3K	Serpina3k ↓
Glucose-6-phosphate 1-dehydrogenase X	G6pdx ↓
Selenium-binding protein 1	Selenbp1 ↓
ATP synthase subunit beta, mitochondrial	Atp5b ↑
Tubulin beta-5 chain	Tubb5 ↓
L-lactate dehydrogenase B chain	Ldhb ↓

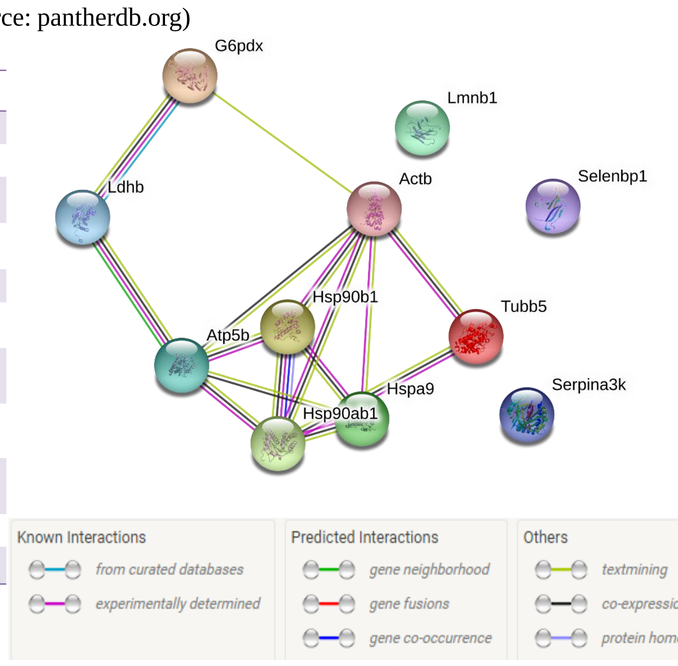


Fig. 3. Proteins which showed differences in expression in the 1st week after cancer cells inoculation (source: string-db.org)

Protein	Gene
Reticulocalbin-1	Rcn1 ↓
EF-hand domain-containing protein D2	Efhd2 ↑
Annexin A5	Anxa5 ↑
Actin, cytoplasmic 1	Actb ↓
14-3-3 protein zeta/delta	Ywhaz ↑
Peroxiredoxin-6	Prdx6 ↓
Chitinase-like protein 4	Chi3l4 ↓
Calumenin	Calu ↓
Rho GDP-dissociation inhibitor 2	Arhgdib ↑
Clathrin light chain A	Clta ↓
Vimentin	Vim ↓
Complement component 1 Q subcomponent-binding protein, mitochondrial	C1qbp ↓

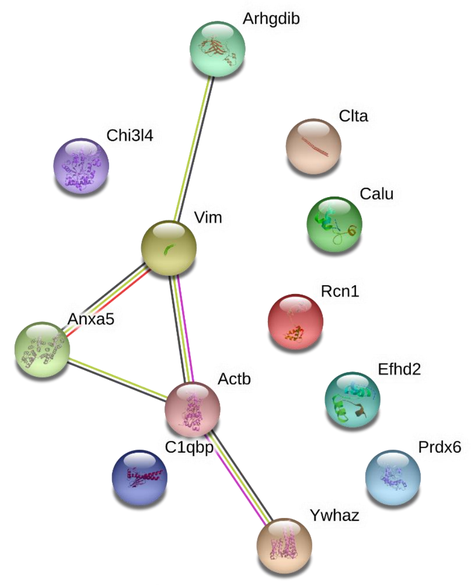


Fig. 4. Proteins which showed differences in expression in the 2nd week after cancer cells inoculation (source: string-db.org)

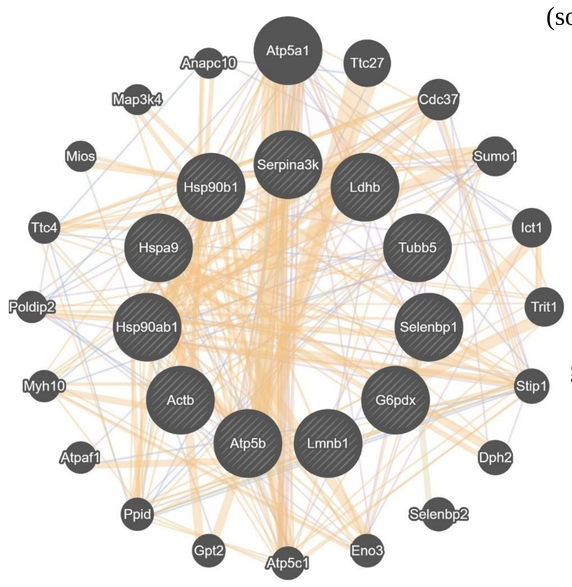
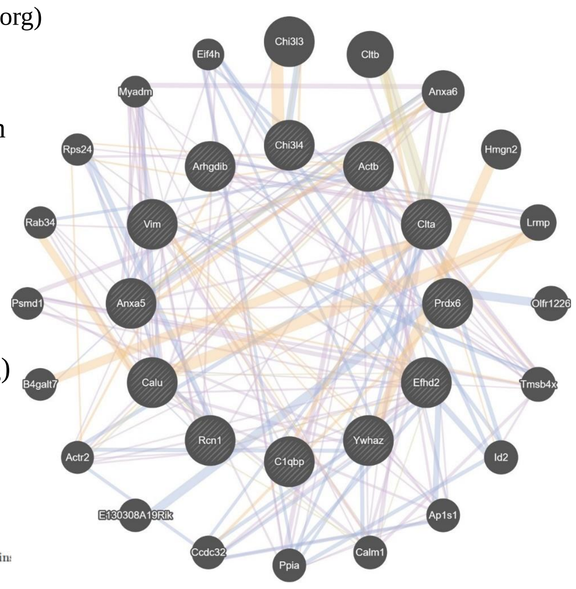


Fig. 5. Other, not detected in the study pottentially differentialy expressed proteins (source: genemania.org)



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