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Department of Biosystems Science and Engineering

Proteomic characterization of the blood-tissue interface of pre-metastatic lung and liver

Experimental Hematology / Oncology

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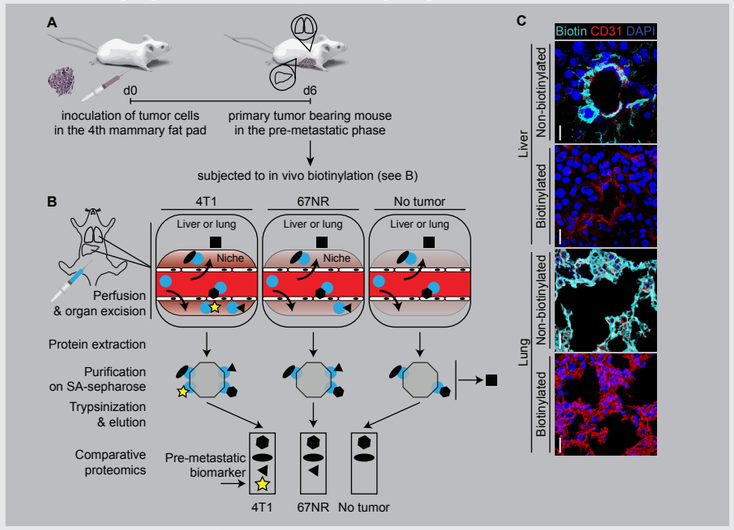
1 Introduction

Metastasis is the primary cause of death in cancer. It has been shown that tumours generate a favourable environment in secondary organs, termed the premetastatic niche, which facilitates the seeding of circulating tumour cells and the formation of metastatic colonies in these new locations. In this study, we employed a chemical proteomics strategy within an orthotopic mouse model of breast cancer, utilising *in vivo* biotinylation of the vasculature, to identify proteins specifically expressed at the blood-tissue interface in premetastatic organs.

2 Methods

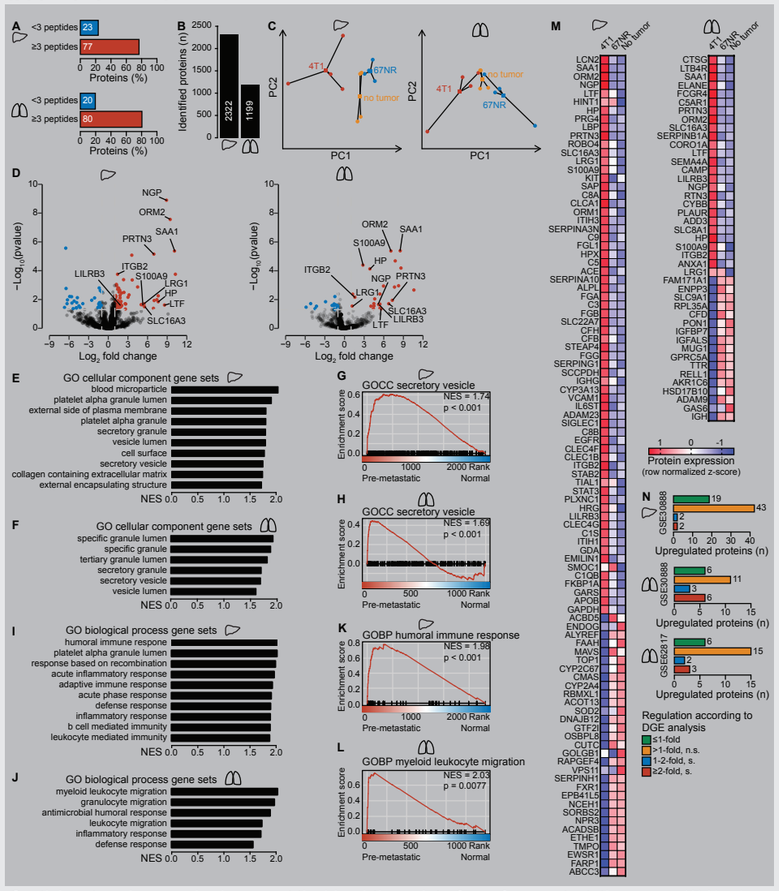
The vasculature of 4T1 tumour-bearing BALB/c mice in the premetastatic phase was perfused with sulfo-NHS-LC-biotin, to covalently label proteins at the blood-tissue border. Matched mice bearing the related but non-metastatic 67NR tumour model and healthy mice served as controls (all n=4). Perfused livers and lungs were harvested, biotinylated proteins purified on streptavidin-resin, and subjected to LC-MS/MS mass spectrometry after tryptic digestion. Resulting spectra were analysed with MaxQuant and protein regulation assessed with Perseus. Targets were validated using immunostaining of murine and human tissue samples. A proteomic dataset on target expression in human normal organs was generated to assess potential on-target side effects of future therapeutics. An *in vivo* biodistribution analysis was performed in mice to assess the specificity of a pharmaceutical drug against the most promising target.



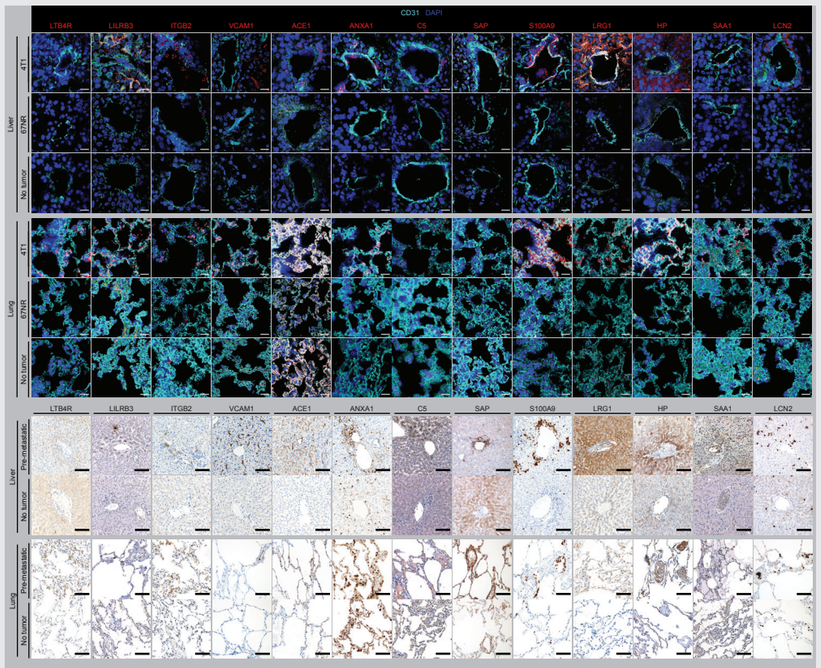
Proteomic characterization of the blood-tissue interface of the pre-metastatic niche. (**A**, **B**) Experimental procedure: BALB/c mice were orthotopically injected with 4T1 cells, 67NR cells or PBS alone. After 6 days, at the pre-metastatic time point, mice were subjected to the *in vivo* protein biotinylation procedure to label vascular and perivascular proteins (black shapes) with biotin. Biotinylated proteins were purified on streptavidin-sepharose and identified and quantified using mass spectrometry. Comparative proteomics reveals biomarkers upregulated at the blood-tissue interface of pre-metastatic organs (yellow star). (**C**) Staining of biotin in relation to the vascular marker CD31. Scale bars are 20 µm.

3 Results

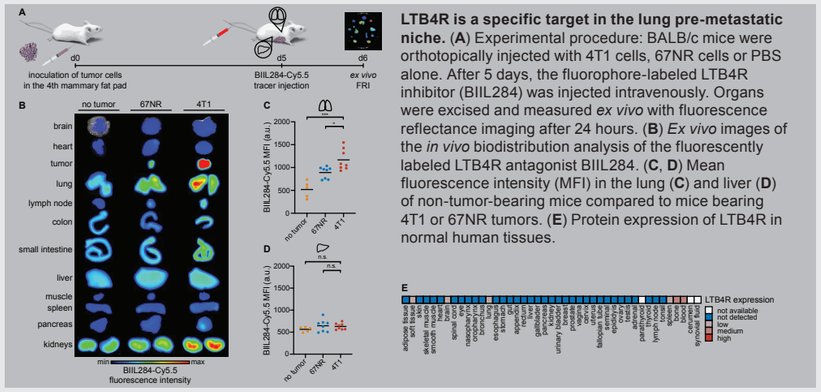
In total, 2322 proteins were identified in the liver and 1199 in the lung. The proteins were mainly related to cellular component gene sets of the cell surface, plasma membrane, extracellular matrix and secretory vesicles. Proteins upregulated in the 4T1 group were mainly related to various inflammatory processes. Interestingly, one protein was identified as a specific biomarker in pre-metastatic lungs. Biodistribution analyses with a fluorophore-tagged clinical-grade inhibitor confirmed it as a potential target of the lung pre-metastatic niche *in vivo*.



Spatial proteomics reveals changes at the blood-tissue interface of pre-metastatic organs. (**A**) Number of proteins identified with <3 vs. ≥3 unique peptides. (**B**) Total number of proteins identified. (**C**) Principal component analysis. (**D**) Volcano plots of the proteomic dataset. Proteins that were upregulated in both, pre-metastatic liver and lung, are indicated. (**E**, **F**, **I**, **J**) Gene set enrichment analysis comparing 4T1 and normal control datasets for liver (**E**, **I**) and lung (**F**, **J**) tissues. (**G**, **H**, **K**, **L**) Enrichment plots of secretory vesicles (**G**, **H**) and the most enriched biological processes (**K**, **L**) in the pre-metastatic liver (**G**, **K**) and lung (**H**, **L**). (**M**) Heatmap of proteins with that were significantly and at least 2-fold regulated between 4T1-bearing and normal mice. (**N**) Number of upregulated proteins that were found or missed by differential gene expression analyses on transcriptomic data from pre-metastatic organs of 4T1 tumor-bearing animals compared to non-tumor-bearing controls (GSE30888, GSE62817)



Upregulation of pre-metastatic biomarkers in the perivascular compartment of the pre-metastatic liver and lung. Representative images of immunostainings of pre-metastatic biomarkers in the murine and human liver and lung. Scale bars are 20 µm and 100 µm respectively.



LTB4R is a specific target in the lung pre-metastatic niche. (**A**) Experimental procedure: BALB/c mice were orthotopically injected with 4T1 cells, 67NR cells or PBS alone. After 5 days, the fluorophore-labeled LTB4R inhibitor (BIIL284) was injected intravenously. Organs were excised and measured *ex vivo* with fluorescence reflectance imaging after 24 hours. (**B**) *Ex vivo* images of the *in vivo* biodistribution analysis of the fluorescently labeled LTB4R antagonist BIIL284. (**C**, **D**) Mean fluorescence intensity (MFI) in the lung (**C**) and liver (**D**) of non-tumor-bearing mice compared to mice bearing 4T1 or 67NR tumors. (**E**) Protein expression of LTB4R in normal human tissues.

5 Conclusion

In conclusion, this comparative spatial analysis of the targetable proteome in the pre-metastatic niche provides a valuable dataset as a basis for the development of pharmaceutical agents targeting tissue remodelling in the pre-metastatic phase of solid tumours.

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