Molecular characterization of single circulating tumor cells from breast cancer patients

Justyna Topa, Laboratory of Cell Biology, Department of Medical Biotechnology, Intercollegiate Faculty of Biotechnology UG and MUG

Supervisor: dr hab. Anna Żaczek

Co-supervisor: dr Aleksandra Markiewicz

Circulating tumor cells (CTCs) are detected in peripheral blood of breast cancer patients and are correlated with poor prognosis. As a result of epithelial-mesenchymal transition (EMT) activation CTCs may show different epithelial-mesenchymal features, stem cell genes expression profile and ability to form metastasis. Thus, EMT is thought to generate CTCs with the most malignant features, which because of loss of epithelial markers are difficult to capture using majority of the methods. Therefore, the knowledge about CTCs characteristics, especially mesenchymal population, is still limited.

The aim of this project is i) to establish single epithelial and mesenchymal CTCs isolation method and ii) to apply it for extensive single cell level phenotype analysis of samples from breast cancer patients. Developed method was based on density gradient centrifugation, negative selection of hematopoietic cells, immunofluorescent staining, single CTCs isolation by micromanipulation and further preamplification allowing for downstream transcriptomic profiling. Immunofluorescent staining of epithelial (EpCAM, E-cadherin) and mesenchymal (cell surface Vimentin, MCAM) markers was optimized in a model spike-in experiment with breast cancer cell lines (MCF-7 and MDA-MB-231), simulating clinical samples. Using this panel of markers it was possible to capture CTCs of both epithelial and mesenchymal phenotypes. To further validate phenotype of single isolated CTCs we used a panel of epithelial (EpCAM, Cytokeratin 19, E-cadherin) and mesenchymal markers (Vimentin, Serpine and Plastin 3), which confirmed CTCs epithelial-mesenchymal state, even those in EMT transitional condition. Next, a panel of genes related to metastasis and stem cell features was examined.

Developed method allows to capture all CTCs phenotypes and perform gene expression analysis on a single cell level. It will allow for detailed characterization of CTCs phenotype, uncovering biological differences underlying aggressive feature of mesenchymal CTCs in clinical samples from breast cancer patients.

***KSZTAŁCIMY NAJLEPSZYCH** – kompleksowy program rozwoju doktorantów, młodych doktorów oraz akademickiej kadry dydaktycznej Uniwersytetu Gdańskiego. Zad. 2. Life Sciences and Mathematics Interdisciplinary Doctoral Studies (LiSMIDoS)*

HUMAN CAPITAL
NATIONAL COHESION STRATEGY

EUROPEAN UNION
EUROPEAN
SOCIAL FUND

Project co-funded by European Union under the European Social Fund