Delineating cellular heterogeneity and organization of breast cancer stem cells

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I. Andersson, D*., **Akrap, N***., Svec, D., Godfrey, T.E., Kubista, M., Göran Landberg, G. and Ståhlberg, A. Properties of targeted preamplification in DNA and cDNA quantification Expert Rev Mol Diagn. 2015 Aug;15(8):1085-100. *Authors contributed equally.

II. **Akrap, N.**, Andersson, D., Gregersson, P., Bom, E., Anders Ståhlberg, A. and Landberg, G. Identification of distinct breast cancer stem cell subtypes based on single cell PCR analyses of functionally enriched stem and progenitor pools. Manuscript.

III. Walsh, C.A., **Akrap, N.,** Magnusson, Y., Harrison, H., Andersson, D., Rafnsdottir, S., Choudhry, H., Buffa, F.M., Ragoussis, J., Ståhlberg, A., Harris, A. and Landberg G. The mevalonate precursor enzyme HMGCS1 is a novel marker and key mediator of cancer stem cell enrichment in luminal and basal models of breast cancer. Manuscript.

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Delineating cellular heterogeneity and organization of breast cancer stem cells

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ABSTRACT

Breast cancer is characterized by a high degree of heterogeneity in terms of histological, molecular and clinical features, affecting disease progression and treatment response. The cancer stem cell (CSC) model suggests, that cancers are organized in a hierarchical fashion and driven by small subsets of CSCs, endowed with the capacity for self-renewal, differentiation, tumorigenicity, invasiveness and therapeutic resistance. The overall aim of this thesis was to characterize CSC phenotypes and the cellular organization in estrogen receptor α + (ERα+) and ERα- subtypes of breast cancer at the individual cell level. Furthermore, we aimed to identify novel functional CSC markers in a subtype-independent manner, allowing for better identification and targeting of breast-specific CSCs.

At present, single-cell quantitative reverse transcription polymerase chain reaction represents the most commonly applied method to study transcript levels in individual cells. Inherent to most single-cell techniques is the difficulty to analyze minute amounts of starting material, which most often requires a preamplification step to multiply transcript copy numbers in a quantitative manner. In **Paper I** we have evaluated effects of variations of relevant parameters on targeted cDNA preamplification for single-cell applications, improving reaction sensitivity and specificity, pivotal prerequisites for accurate and reproducible transcript quantification.

In **Paper II** we have applied single-cell gene expression profiling in combination with three functional strategies for CSC enrichment and identified distinct CSC/progenitor clusters in ERα+ breast cancer. ERα+ tumors display a hierarchical organization as well as different modes of cell transitions. In contrast, ERα- breast cancer show less prominent clustering but share a quiescent CSC pool with ERα+ cancer. This study underlines the importance of taking CSC heterogeneity into account for successful treatment design.

In **Paper III** we have used a non-biased genome-wide screening approach to identify transcriptional networks specific to CSCs in ERα+ and ERα- subtypes. CSC-enriched models revealed a hyperactivation of the mevalonate metabolic pathway. When detailing the mevalonate pathway, we identified the mevalonate precursor enzyme 3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1) as a specific marker of CSC-enrichment in ERα+ and ERα- subtypes, highlighting HMGCS1 as a potential gatekeeper for dysregulated mevalonate metabolism important for CSC-features. Pharmacological inhibition of HMGCS1 could therefore be a novel treatment approach for breast cancer patients targeting CSCs.

Keywords: Breast cancer, cancer stem cells, cellular heterogeneity
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