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Identification and Characterization of a Proliferative Cell Population in Estrogen Receptor-Positive Metastatic Breast Cancer through Spatial and Single-Cell Transcriptomics

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Background. We applied spatial transcriptomics and single-cell RNA-sequencing on estrogen-responsive patient-derived xenografts (PDXs) to profile spatially resolved cell populations within estrogen receptor-positive (ER^+) metastatic breast cancer and elucidate their importance in estrogen-dependent tumor growth. **Methods.** Two PDXs of "ER-high" metastatic breast cancers with opposite estrogen-mediated growth responses were studied: estrogen-suppressed GS3 (80–100% ER) and estrogen-stimulated SC31 (30–75% ER) models. The observation was validated via single-cell analyses on another "ER-low" estrogen-accelerating PDX, GS1 (5% ER). The results from our spatial and single-cell analyses were further supported by a public ER^+ breast cancer single-cell dataset and protein-based dual immunohistochemistry (IHC) using important clinical markers [i.e., ER, progesterone receptor, and Ki67]. The translational implication of our findings was assessed by clinical outcome analyses on public breast cancer cohorts. **Results.** Our space-gene-function study revealed four spatially distinct compartments within ER^+ metastatic breast cancers. These compartments showed functional diversity (estrogen-responsive, proliferative, hypoxia-induced, and inflammation-related). The "proliferative" population, not "estrogen-responsive" compartment, was crucial for estrogen-dependent tumor growth, leading to the acquisition of luminal B features. The cells inducing typical estrogen-responsive genes like *PGR* were not directly linked to estrogen-dependent proliferation. The IHC analyses demonstrated the distinct contribution of the *Ki67*$^+$ proliferative cells toward estrogen-mediated growth and their response to a CDK4/6 inhibitor. The gene signatures developed from the proliferative, hypoxia-induced, and inflammation-related compartments were significantly correlated with worse clinical outcomes, while patients with the estrogen-responsive signature showed better prognosis, confirming that this compartment would not be directly associated with estrogen-dependent tumor progression. **Conclusions.** Our study elucidated the gene signature in our "proliferative" compartment as an important determinant of luminal cancer subtypes. This "proliferative" cell population is a causative feature of luminal B metastatic breast cancer, contributing toward its aggressive features.