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Identification of Distinct Breast Cancer Stem Cell Populations Based on Single-Cell Analyses of Functionally Enriched Stem and Progenitor Pools

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SUMMARY

The identification of breast cancer cell subpopulations featuring truly malignant stem cell qualities is a challenge due to the complexity of the disease and lack of general markers. By combining extensive single-cell gene expression profiling with three functional strategies for cancer stem cell enrichment including anchorage-independent culture, hypoxia, and analyses of low-proliferative, label-retaining cells derived from mammospheres, we identified distinct stem cell clusters in breast cancer. Estrogen receptor (ER)α+ tumors featured a clear hierarchical organization with switch-like and gradual transitions between different clusters, illustrating how breast cancer cells transfer between discrete differentiation states in a sequential manner. ERα− breast cancer showed less prominent clustering but shared a quiescent cancer stem cell pool with ERα+ cancer. The cellular organization model was supported by single-cell data from primary tumors. The findings allow us to understand the organization of breast cancers at the single-cell level, thereby permitting better identification and targeting of cancer stem cells.

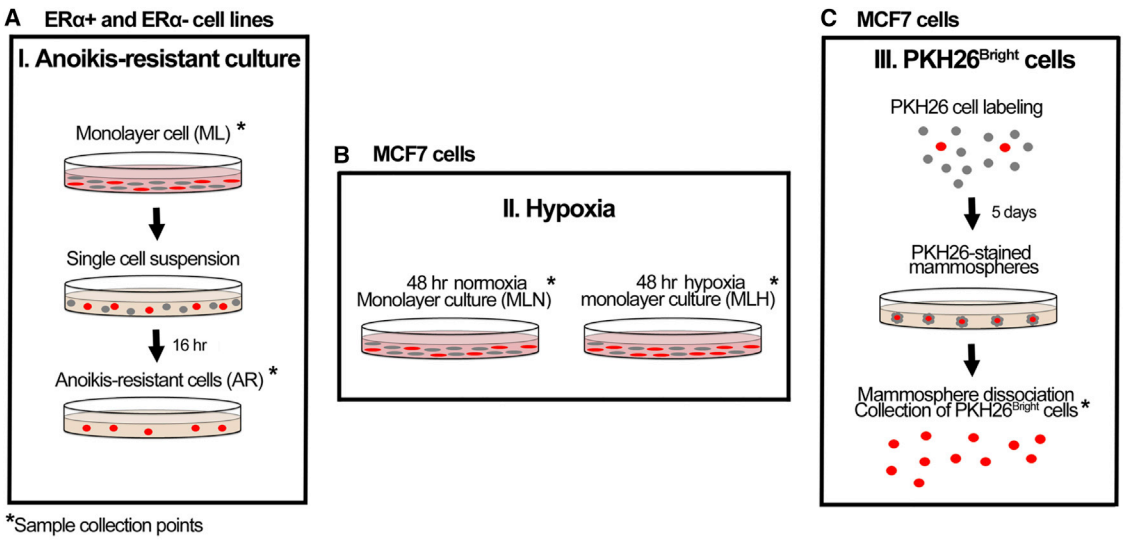
INTRODUCTION

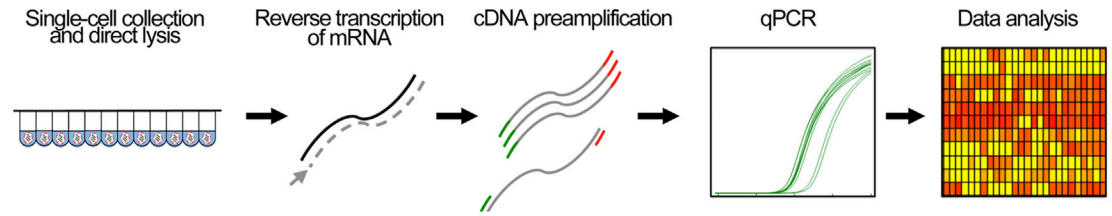
Breast cancer is one of the world's leading causes of cancer-related death among women, characterized by a high degree of heterogeneity in terms of histological, molecular, and clinical features, affecting disease progression and treatment response (Bertos and Park, 2011). This has led to the classification of breast cancer into several subtypes including classical histological and immunohistochemical definitions of breast cancer types as well as molecularly defined subgroups (Perou et al., 2000; Sørlie et al., 2001). The seminal studies by Perou et al. and Sørlie et al. identified luminal, HER2-enriched, basal, and normal-breast-like intrinsic breast cancers. At the transcriptomic level, this classification was shown to be mainly driven by estrogen receptor α (ERα), and ERα-related and proliferation-related genes (Reis-Filho and Pusztai, 2011). ERα-positive (ERα+) and -negative (ERα−) breast cancers are well recognized as molecularly and clinically distinct diseases. Several hypotheses have been proposed to explain intertumoral heterogeneity; including different genetic and epigenetic aberrations as well as distinct subtype-specific tumor cells of origin (Polyak, 2011).

Functional and phenotypic diversity has also been described at the single-cell level within individual tumors. Cells of various cancer types have been shown to differ greatly in their tumorigenic, angiogenic, invasive, and metastatic potential (Polyak, 2011). To account for intratumoral heterogeneity the cancer stem cell (CSC) model suggests that tumors are driven by a cellular subpopulation with stem cell properties, giving rise to hierarchically structured tumors. Attributes of CSCs comprise self-renewal, tumorigenicity, multilineage differentiation, and increased resistance to radiotherapy- and chemotherapy-induced cell death (Badve and Nakshatri, 2012), making CSCs critical targets in cancer therapy.

CSCs of breast tumors are commonly enriched by combinations of several cell-surface antigens, such as CD44/CD24/EPCAM (Al-Hajj et al., 2003), or by high ALDH (aldehyde dehydrogenase) activity (Ginestier et al., 2007). However, existing markers lack specificity, also reflective of a substantial proportion of non-CSCs. Furthermore, the applicability of existing markers is often limited to specific breast cancer subtypes (Nakshatri et al., 2009) in addition to interindividual intrinsic differences (Visvader and Lindeman, 2012). Previous studies have investigated the CSC content in different breast cancer subtypes (Harrison et al., 2013; Kim et al., 2012; Ricardo et al., 2011); however, thus far it is not exactly known whether distinct subtypes harbor the same or dissimilar CSCs. The large multitude of assays currently employed indicates either a lack of universal markers or reflects the heterogenic and dynamic nature of CSCs. The exact characterization of putative CSC pools is a pivotal requirement for clinical identification, monitoring, and targeting of these cells.

To elucidate the heterogeneity of the CSC pool and to study the CSC compartment in ERα+ and ERα− breast cancer subtypes, we set up a single-cell quantitative real-time PCR (qPCR) approach, profiling the expression of well-established key regulators involved in differentiation,





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Gene Group	Gene	References
Epithelial/Differentiation	CDH1	Singhai *et al.*, 2011; Younis *et al.*, 2007
	CD24	Al-Hajj *et al.*, 2003
	EPCAM	Al-Hajj *et al.*, 2003; Pece *et al.*, 2010
	ESR1	Predictive and prognostic marker, Visvader, 2009
	PGR	Predictive and prognostic marker, Visvader, 2009
Breast Cancer Stem Cell	CD44	Al-Hajj *et al.*, 2003
	ITGA6	Cariati *et al.*, 2008
	DNER	Pece *et al.*, 2010
	ALDH1A3	Ginestier *at al.*, 2007; Charafe-Jauffret *et al.*, 2009, Marcato *et al.*, 2011
	ABCG2	Doyle *et al.*, 1998
Pluripotency	POU5F1	Ben-Porath *et al.*, 2008: Prud'homme, 2012
	NANOG	Ben-Porath *et al.*, 2008; Prud'homme, 2012
	SOX2	Ben-Porath *et al.*, 2008; Prud'homme, 2012
EMT/Metastasis	SNAI1	Mani *et al*, 2008; de Herreros *et al.*, 2010; Smith *et al.*, 2014
	SNAI2	de Herreros *et al.*, 2010
	FOSL1	Lu *et al.*, 2012; Desmet *et al.*, 2013;
	VIM	Vuoriluoto *et al.*, 2011; Liu *et al.*, 2015
	CDH2	Nieman *et al.*, 1999; Chung *et al.*, 2013
	ID1	Schoppmann *et al.*, 2003; Gupta *et al.*, 2007; Gumireddy *et al.*, 2014
Proliferation	CCNA2	Well-established proliferation marker
	MKI67	Well-established proliferation marker
	ERBB2	Predictive and prognostic marker, Visvader, 2009

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