Abstract # 4317

The sialyl-glycolipid SSEA4 marks a subpopulation of chemotherapy resistant breast cancer cells with mesenchymal features

Miltenyi Biotec
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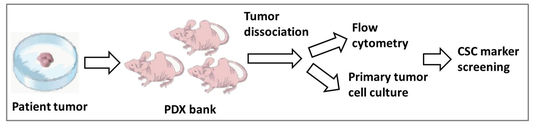
Currently, the main option for systemic therapy of high-risk breast cancer is chemotherapy, with an overall poor efficacy and severe side effects. Chemotherapy-resistance and incomplete pathologic response associate with risk of metastasis and early relapse in breast cancer.

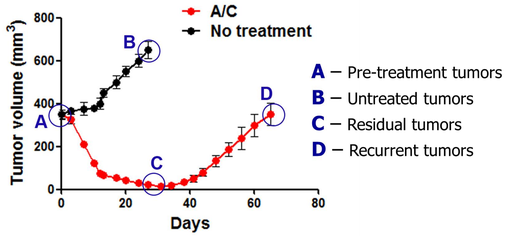
In order to characterize treatment-resistant tumor cells, we performed a cell surface marker screen in 4 triple-negative breast cancer patient-derived xenograft (PDX) models that respond well to adriamycin/cyclophosphamide-based chemotherapy but fail to reach complete pathological response. We used multi-parameter flow cytometry to screen for the expression of a set of 45 cell surface markers during the course of chemotherapy. This set of markers represented both proteins involved in stem cell function and proteins known to be over-expressed in stem cells or cancer stem cell sub-populations.

Identification of cellular biomarkers of chemo-resistance

PDX tumor bank as a source of human breast cancer tissue

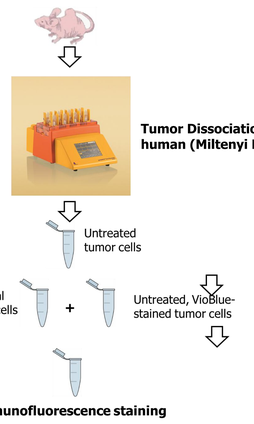


In vivo induction of PDX tumor regression and re-growth with adriamycin / cyclophosphamide chemotherapy

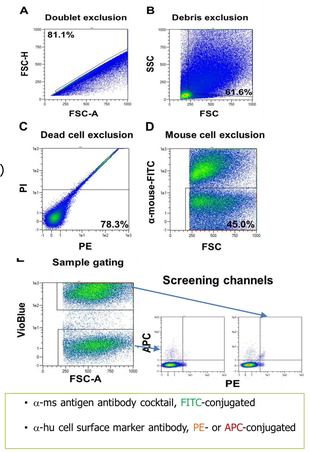


Cell surface marker antibody library: a tool to search for a better marker

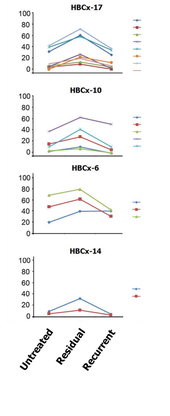
Marker ID (antibodies)	Gene symbol	Description	Marker ID (antibodies)	Gene symbol	Description
ABCB5	ABCB5	ATP-binding cassette, sub-family B of integral membrane proteins	**CD34**	CD34	highly glycosylated single-pass membrane protein
AN2/MCSP	CSPG4	an integral membrane chondroitin sulfate proteoglycan	**CD340 (HER2/neu)**	ERBB2	receptor tyrosine kinase EGF family
CaSR	CASR	calcium-sensing receptor	**CD38**	CD38	ectoenzyme
CD10	MME	a common acute lymphocytic leukemia antigen	**CD44**	CD44	cell-surface glycoprotein, receptor for hyaluronic acid
CD105 (Endoglin)	ENG	homodimeric transmembrane glycoprotein endoglin	**CD49a**	ITGA1	alpha 1 subunit of integrin receptors
CD117	KIT	type 3 transmembrane receptor for mast cell growth factor	**CD49b**	ITGA2	integrin, alpha 2 subunit
CD122	IL2RB	interleukin 2 receptor, beta	**CD49c**	ITGA3	integrin, alpha 3 subunit
CD133/1	PROM1	prominin 1, a pentaspan transmembrane glycoprotein	**CD49d**	ITGA4	integrin, alpha 4 subunit of VLA-4 receptor
CD133/2	PROM1		**CD49e**	ITGA5	integrin, alpha 5 subunit of fibronectin receptor
CD138	SDC1	syndecan, transmembrane (type I) heparan sulfate proteoglycan	**CD49f**	ITGA6	integrin, alpha 6 subunit
CD146	MCAM	melanoma cell adhesion molecule	**CD61**	ITGB3	integrin, beta 3 subunit
CD15/SSEA1	FUT4	fucosyltransferase 4	**CD66 (a,c,d,e)**	CEACAM1	carcinoembryonic antigen-related cell adhesion molecule 1
CD166	ALCAM	activated leukocyte cell adhesion molecule	**CD71**	TFRC	transferrin receptor
CD20	MS4A1	membrane-spanning 4-domains, subfamily A, member 1	**CD9**	CD9	cell surface glycoprotein, tetraspanin protein family
CD24	CD24	cell surface sialoglycoprotein	**CD90**	THY1	Thy-1 cell surface antigen
CD26	DPP4	membrane glycoprotein	**DRD5**	DRD5	dopamine receptor D5
CD271	NGFR	nerve growth factor receptor	**Lgr5 DA03**	LGR5	leucine-rich repeat containing G protein-coupled receptor 5
CD309	KDR	kinase insert domain receptor tyrosine kinase	**ROR1**	ROR1	receptor tyrosine kinase-like orphan receptor 1
CD324-Ecad	CDH1	E-cadherin, a calcium dependent cell-cell adhesion glycoprotein	**SSEA4**	-	Sialylgalactosylgloboside, stage-specific embryonic antigen 4
CD325-Ncad	CDH2	N-cadherin, a calcium dependent cell-cell adhesion glycoprotein	**TGFbetaR**	TGFBR1	a serine/threonine protein kinase
CD326	EPCAM	epithelial cell adhesion molecule	**TRA-1-60**	-	a cell surface antigen on undifferentiated human EC cells
CD338	ABCG2	ATP-binding cassette, sub-family G (WHITE), member 2	**TRA-1-81**	-	Epitope associated with a keratan-sulfated transmembrane protein



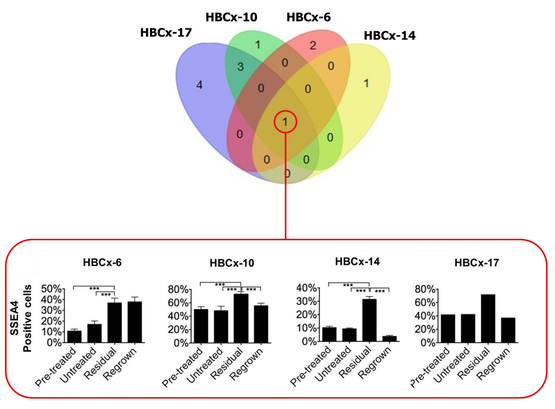
Gating strategy and evaluation of cell surface marker expression by flow cytometry-based analysis



Marker enrichment



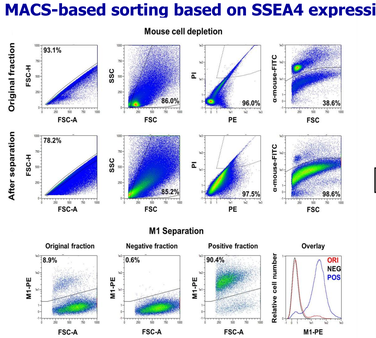
SSEA4 is enriched in residual tumor cells from all models



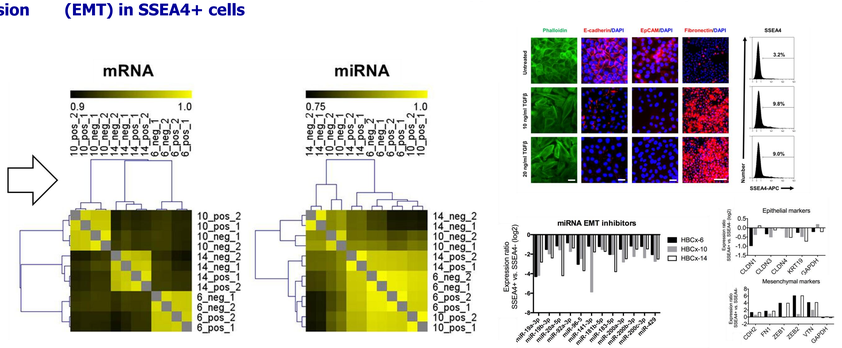
We identified the sialyl-glycolipid SSEA4 as a constant marker of chemotherapy-resistant cancer cells in all four models. In addition, SSEA4 expression was found higher in 3 out of 4 TNBC PDXs that are de novo resistant to neo-adjuvant chemotherapy compared to sensitive TNBC PDXs. Two cell populations with different percentage of SSEA4-positive (SSEA4+) cells and with different growth characteristics were identified in a PDX model. When treated with genotoxic compounds, the cell population with higher SSEA4+ expression showed increased resistance to chemotherapy, indicating this post-translational modification as potential marker of tumor resistance. Comparison of SSEA4+ and SSEA4-negative (SSEA4-) tumor cells from TNBC PDX models by global gene expression profiling showed overexpression of mesenchymal-associated genes in SSEA4+ tumor cells and a deregulation of drug resistance pathway-associated genes and miRNAs. In addition, high expression of ST3 beta-galactoside alpha-2,3-sialyltransferase 2 (ST3GAL2), the enzyme catalyzing the last step of SSEA4 synthesis, was found associated with poor outcome in breast and ovarian cancer patients treated with chemotherapy.

Molecular and functional analyses of cells sorted according to SSEA4 expression show EMT phenotype and increased resistance to genotoxic treatment in SSEA4-positive cells

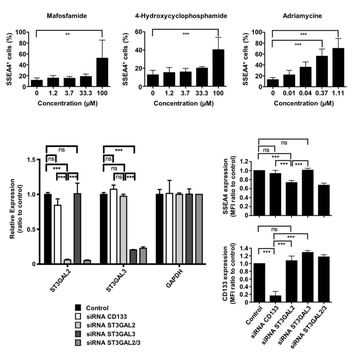
Flow cytometry analysis of PDX tumor cells by MACS-based sorting based on SSEA4 expression

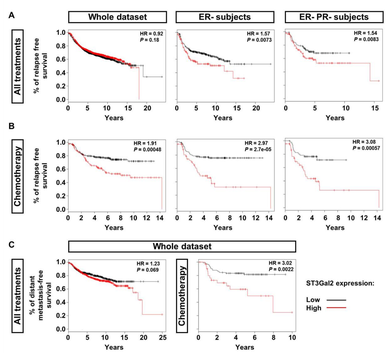


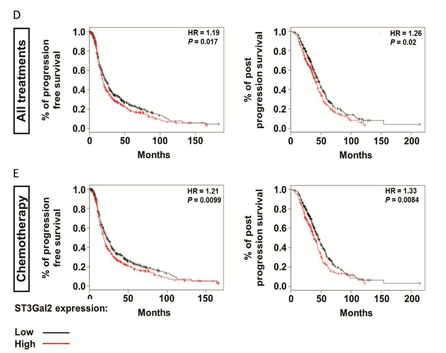
Molecular profiling reveals loss of miRNAs that suppress epithelial-mesenchymal transition (EMT) in SSEA4+ cells



SSEA4-positive cells are enriched upongenotoxic stress *in vitro*, and expression of ST2GAL3, which catalyzes SSEA4 synthesis, stratifies patients according to tumor response to chemotherapy and overall survival in breast (A-C) and ovarian (D-E) cancer







Thus, we propose SSEA4 as a novel marker of epithelial-mesenchymal transition associated with chemoresistance, and ST3GAL2 expression as a predictive marker for tumor chemoresistance associated with poor outcome in breast and ovarian cancer patients. Both biomarkers and additionally identified regulatory miRNAs may be used to further understand chemoresistance and to develop alternative treatment regimens for breast and ovarian cancer patients.