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EXPRESSION PROFILING OF CLINICAL SPECIMENS SUPPORTS THE EXISTENCE OF NEURAL PROGENITOR-LIKE STEM CELLS IN BASAL BREAST CANCERS

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Abstract

To facilitate development of CSC-targeting therapies, we analyzed publicly available breast cancer datasets to identify genes co-expressed with SOX10, a neural crest marker, and PROM1/CD133, a common CSC marker. The conserved SOX10/PROM1 gene signature that we characterized supports the existence in basal breast cancers of SOX10+/CD133+ cells with neural stem-like features exposing clinically relevant CSC markers and signaling networks.

Background—We previously characterized in salivary adenoid cystic carcinoma (ACC) a novel population of CSC marked by co-expression of two stemness genes, SOX10 and CD133. We also reported that in ACC and basal-like breast carcinoma (BBC), a triple-negative breast cancer subtype, expression of SOX10 similarly demarcates a highly conserved gene signature enriched with neural stem cell genes. Based on these findings, we hypothesized that BBC may be likewise driven by SOX10+/CD133+ cells with neural stem cell properties.

Methods—To validate our hypothesis on clinical data, we used a novel approach to meta-analysis that merges gene expression data from independent breast cancer studies and ranks genes by statistical significance of their co-expression with the gene of interest. Genes that showed strong association with both CD133/PROM1 and SOX10 were validated across different platforms and datasets and analyzed for enrichment with genes involved in neurogenesis.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interests.

Results—We identified in clinical breast cancer datasets a highly conserved SOX10/PROM1 gene signature that contains neural stem cell markers common for Schwann cells, ACC, BBC, and melanoma. Identification of TRIM2, TRIM29, MPZL2, KCNN4, and VTCN1/B7-H4 within this signature provides insight into molecular mechanisms of CSC maintenance.

Conclusion—Our results suggest that BBC is driven by SOX10+/CD133+ cells that express NSC-specific markers and share molecular similarities with CSC of neural crest origin. Our study provides clinically relevant information on possible drivers of these cells that may facilitate development of CSC-targeting therapies against this cancer distinguished with poor prognosis and resistance to conventional therapies.

Keywords

SOX10; CD133; basal-like breast carcinoma; triple-negative breast cancers; adenoid cystic salivary carcinoma; cancer stem cells; neural stem cells; FABP7; TRIM2; TRIM29; MPZL2; KCNN4; MPZL2; B7-H4

BACKGROUND

CSC are attractive therapeutic targets due to their abilities to maintain tumor growth, invade, metastasize, withstand cytotoxic therapies, and recur¹. However, isolation of CSC from tumor specimens is challenging, especially when information on clinically relevant cell surface markers is lacking. The great majority of published CSC studies relies on *in vitro* studies, but significance of CSC markers derived from cell lines is questionable, especially when their expression in clinical specimens is not confirmed^{2–6}. We believe that molecular profiling of clinical specimens could help the pursuit for clinically relevant CSC. If CSC with similar properties are shared across tumor specimens, such cells can be tracked by gene expression patterns conserved between patients. It can be also argued that stemness markers related to cell of origin can be shared by cancers with similar histogenesis and exposed via gene expression profiling. A remarkable example of such a marker is SOX10 (SRY-related HMG box-containing factor 10), which plays pivotal roles in gliogenesis, melanogenesis, differentiation of myoepithelial cells and other lineages that originate from neural crest, and maintenance of adult neural stem cells (NSC) that reside in the subventricular CNS zone^{7–12 8, 13–15}. Recent studies that characterized SOX10 as a melanoma driver¹⁶, and a key breast stem cell regulator¹⁷ raised the important question about SOX10 relevance to stem cell maintenance in a certain group of cancers.

Our previously performed expression analysis of clinical specimens of adenoid cystic carcinoma (ACC), a highly lethal neuroinvasive cancer of salivary glands, identified in 94% of ACC transcripts of SOX10, NOTCH1, FABP7, and other NSC genes¹⁸ whose co-expression was remarkably consistent from patient to patient. The most plausible explanation for this phenomenon was the existence in the majority of ACC of a previously uncharacterized population of CSC cells with NSC properties. Indeed, our recent experiments performed on cultured ACC cells and PDX models demonstrated that SOX10 marks a previously unrecognized population of CD133+ stem cells that express stemness genes and form tumors with ACC histology when injected into nude mice¹⁹. Overall, these experiments validated SOX10 as a valuable cell lineage marker and key ACC-CSC regulator

suggesting that identification of genes associated with SOX10 activation may provide important insights into molecular features of previously uncharacterized CSC in SOX10-positive cancers.

Interestingly, we and others previously demonstrated that SOX10 expression is also diagnostic for basal-like breast carcinoma (BBC), a breast cancer subtype distinguished with myoepithelial-like gene expression profile and myoepithelial differentiation akin to ACC^{8, 13, 14, 17}. BBC as a triple-negative breast cancer (TNBC) subtype was described 15 years ago based on a characteristic gene expression signature found in ~15% of breast cancer specimens^{20–22}. This finding was the first molecular insight into BBC that urgently requires targeted therapies due to the absence of estrogen, progesterone, and ERBB2 receptors, shows poor prognosis, and affects young women²³. Based on the observed similarities between ACC and BBC, we hypothesized that SOX10 promotes in both cancers CD133+ cells with stem cell properties that could be tracked in clinical tumor specimens via genes that are co-expressed with CD133 and SOX10.

Tracking conserved elements of gene expression patterns across multiple specimens and datasets requires a reliable noise-resistant and straightforward algorithm for meta-analysis. Here, using Multi Experiment Matrix (MEM) meta-analysis that merges information from different Affymetrix datasets into a single global ordering and ranks genes by their co-expression²⁴, we confirm that CD133 and SOX10 co-expression in breast cancers is highly statistically significant, intrinsic to BBC specimens, and clinically and biologically relevant. This observation supports our hypothesis on the existence in BBC of SOX10+/CD133+ cells with neural stem properties and provides a blueprint for identification of true CSC and their targeting.

MATERIALS AND METHODS

Meta-analysis

of publicly available breast cancer datasets was performed using Multi Experiment Matrix (MEM) software and datasets produced on Affymetrix U133 plus 2.0 platform. MEM ranks Affymetrix probes/genes by statistical score assigned based on co-expression of these probes with the gene of interest across multiple datasets. To improve biological and clinical aspects of the analysis, MEM allows access to individual datasets, performs stratification of output data, and builds heat maps to identify clinical groups with differential gene expression^{24, 25}.

Cross-validation

of meta-data was done with cBioPortal software on a TCGA Breast Invasive Carcinoma dataset at http://www.cbioportal.org/study?id=brca_tcga#summary. This dataset was produced on 1105 clinical specimens collected from 1098 patients and combines mutational analysis with DNA copy number variation, gene expression array, and RPPA data.

Breast cancer PDX models

were profiled using E-GEOD-46106 dataset and software available at BioGPS (www.biogps.org). This dataset was produced using U133 Plus 2.0 Affymetrix platform on xenografts established from 25 breast cancer patients.

Analysis of discriminators

for breast cancer groups was done via <http://www.ncbi.nlm.nih.gov/geo/geo2r/>, a web tool available via Gene Expression Omnibus for comparative statistical analysis between two or more groups for identification of differentially expressed genes.

Gene ontology analysis

was done via <http://software.broadinstitute.org/gsea/>, a computational method that determines whether a priori defined sets of genes show statistically significant, concordant differences between two biological states (e.g. phenotypes).

Gene expression analysis of human primary cells

was performed using Primary Cell Atlas dataset (http://biogps.org/dataset/BDS_00013/) and software available at BioGPS. This dataset combines 745 samples from over 100 studies performed on human primary cells.

RESULTS

Expression of PROM1/CD133 in breast cancers strongly correlates with SOX10, its core signature elements, and BBC-intrinsic stem cell markers

We previously demonstrated that PROM1/CD133 is co-expressed with SOX10 in the great majority of clinical ACC specimens and in a novel population of SOX10+/CD133+ stem cells, ACC-CSC, that we recently characterized¹⁹. Furthermore, we showed that BBC is molecularly similar to ACC as it expresses SOX10 with its core gene signature elements ROPN1, ROPN1B, TRIM2, and GPM6B^{14, 26–28} suggesting that BBC may contain a similar population of SOX10+/CD133+ cells with neural stem cell properties. Here, we interrogated publicly available clinical breast cancer datasets to determine if PROM1/CD133 expression is also BBC-intrinsic and correlates with SOX10 expression. Using MEM server (<http://biit.cs.ut.ee/mem/>) that uses a novel statistical rank aggregation algorithm that ranks genes by their co-expression with the gene of interest²⁵, we analyzed 23 publicly available datasets to identify genes whose expression closely correlates with PROM1. In line with our expectation, SOX10 and core SOX10 signature genes ROPN1, ROPN1B, TRIM2, and GPM6B¹⁴ showed statistically significant association with PROM1 across these 23 datasets (FIG. 1). We then asked if this PROM1 gene signature contains previously published BBC/TNBC markers. Remarkably, we found that almost half (19/41) of genes with highest p values ($e-50 < p < e-28$) have been previously linked to BBC (FIG. 1, blue font). Interestingly, GABRP, which encodes the enigmatic π subunit of gamma-aminobutyric acid type A receptor, has been previously linked to CD133-expressing hepatic progenitors²⁹ and reported as a BBC marker in several studies^{30–32}. VGLL1, one of four human orthologs of *Drosophila* vestigial, is an intriguing but poorly studied component of Wnt and TEAD

signaling whose expression in BBC was previously linked to CD133/PROM1 and GABRP^{30, 33}. While VGLL1 relation to stem cell maintenance in BBC remains unclear, its paralog VGLL4 promotes survival of human embryonic stem cells³⁴ and regulates Hippo-YAP signaling in postnatal cardiomyocytes³⁵. Other previously published BBC markers that we found in the PROM1 signature were transcription factors ELF5, BCL11A, and SOX9, all of them linked to promotion of progenitor/stem cells in breast cancer and normal breast^{36–39}, and CDH3/P-cadherin, a breast basal stem cell marker⁴⁰. Thus, our analysis of the PROM1/CD133 MEM signature was consistent with the existence in BBC, but not other breast cancer types, SOX10+/CD133+ cells that are molecularly similar to ACC-CSC and express stem cell genes previously linked to both ACC and BBC.

CD133 expression in breast cancer is BBC-selective

Our hypothesis that the CD133/PROM1 gene signature is BBC-selective was then validated on one of the 23 datasets used in the MEM analysis, E-GEOD-21653, which stratified clinical specimens into five major groups: ERBB2, BBC, luminal A, luminal B, and normal-like subtypes (N total=266). The heat map built for this study at <http://biit.cs.ut.ee/mem/> revealed that PROM1 is strongly and consistently expressed in the great majority of BBC (~80%), in a fraction of ERBB2+ tumors, and in the normal-like tumor group, but to a much lesser extent in the luminal subtypes (FIG. 2). In line with this finding, CD133/PROM1 was among top 250 discriminators of the BBC subtype in our analysis of distribution of gene expression across five breast cancer groups (<http://www.ncbi.nlm.nih.gov/geo/geo2r/>, $P_{\text{adj}} = 7.7\text{E-}29$). We also observed expression of PROM1 in normal-like breast specimens included in E-GEOD-21653 (FIG. 2) as well as in normal breast specimens represented in other datasets that we analyzed (data not shown), which was in line with previously published reports on PROM1 expression in normal breast⁴¹. Interestingly though, some PROM1 signature genes, such as ROPN1, ROPN1B, MPZL2, GRHL1, LCN2, MIA, KLF5, FOXC1, PERP, and VGLL1 (shown with arrows in FIG. 2), appeared to be more selective to cancerous tissue than PROM1. Overall, our analysis strongly supports a major role for SOX10+/CD133+ cells in BBC providing a set of novel molecular markers for these prospective CSC and a new insight into signaling pathways involved in their maintenance.

Cross-validation of the PROM1 gene signature

As MEM rank aggregation analysis has not been previously used for CSC tracking, we validated our PROM1 gene signature on a large breast cancer dataset (Breast Invasive Carcinoma, TCGA Provisional, N=1093, RNA-Seq and RPPA data available from <http://www.cbioportal.org>). In this dataset, statistically significant associations found between SOX10, PROM1, and core SOX10 signature genes supported the results of our MEM analysis (Table 1). Using standard Pearson and Spearman score calculation, a list of genes positively and negatively correlating with PROM1 in the TCGA dataset was generated (r cutoff 0.5, Suppl. Table 1, N=197) and compared with the MEM-generated PROM1 gene signature shown in FIG. 1. Remarkably, 80% of these PROM1 signature genes (33 of 41) were reproduced in this validation study as positively correlating with PROM1 (Suppl. Table 1, grey rows). On the other hand, estrogen receptor (ESR1, $r=-0.62$), as well as FOXA1 ($r=-0.55$) and GATA3 ($r=-0.56$), two transcription master regulators and molecular markers of

sex hormone receptor-positive breast cancers⁴², were at the very bottom of the list of genes with the highest negative correlation with PROM1.

We then used RPPA data available with the TCGA dataset and demonstrated that PROM1-expressing specimens were mostly ESR1, PGR, and ERBB2/HER2-negative ($e-10 < p < e-27$) and overexpressed NOTCH1 ($p \sim e-7$), CDH3 ($p \sim e-3$), and previously published BBC markers (Suppl. Table 2).

Overall, cross-validation of our MEM meta-analysis on an independent cohort at both RNA and protein levels strengthened clinical relevance of our findings and produced new information of their molecular identity.

Identification of TRIM2, TRIM29, MPZL2, KCNN4, and VTCN1/B7-H4 within the PROM1 gene signature suggests strategies for CSC eradication

Within the PROM1 signature, we found genes that, to our knowledge, have not been previously associated with BBC/TNBC (FIG. 1, red font). Two of these genes were represented by TRIM proteins implicated in neurodegenerative disorders, axonopathies and ataxia^{43–45}. The TRIM2 function in cancers has been associated with its E3 ubiquitin ligase activity towards BIM/BCL2L11, its pro-apoptotic substrate^{46, 47}. Pro-tumorigenic activities of TRIM29 appear to be mediated via multiple mechanisms as its overexpression in cancers was linked with suppression of p53, PTEN, and promotion of radioresistance and other stem cell properties^{48–51}. Other potentially useful therapeutic targets within the PROM1 signature were MPZL2/EVA1, which has been recently linked to maintenance of glioblastoma stem cells through activation of non-canonical NFκB pathway⁵², and KCNN4 that encodes potassium channel KCa3.1 involved in regulation of motility in glioblastoma stem cells⁵³. One of the most intriguing genes that we associated with PROM1 expression was VTCN1, which encodes B7-H4, a checkpoint receptor in immune tolerance regulation⁵⁴. Interestingly, expression of B7-H4 has been previously demonstrated in CSC in neuroectodermal tumors and linked to stimulation of CSC in renal cell carcinoma where it was essential for tumor growth, chemoresistance, and immune evasion^{55, 56}. Supporting their clinical relevance, all these five genes passed cross-validation on the TCGA dataset (Suppl. Table 1). Thus, tracking CSC in clinical specimens by their molecular markers may provide valuable information on genes and signaling pathways intrinsically linked to poor prognosis, which may facilitate development of CSC-targeting therapies for BBC and other cancers.

Expression of PROM1 in BBC is paralleled by activation of neural stem genes

Our previous studies associated SOX10 and CD133 expression in ACC with expression of neural stem cell genes and markers^{14, 18, 19}. To find out if this is also true for BBC, we performed enrichment analysis of genes listed in Suppl. Table 1. Our GEO analysis performed using software available from <http://software.broadinstitute.org/gsea/index.jsp> revealed that the list of 149 genes co-expressed with PROM1 show has highly statistically significant overlaps with CNS Development and Neurogenesis GO sets (Suppl. Table 3). SMO, ID4, FABP7, NFIB, FZD9, CX3CL1, and LMO4 that have been previously

characterized as key signaling factors involved in NSC regulation were the most interesting genes in these overlaps with therapeutic potential^{57–63}.

Schwann cell markers reveal similarities between BBC and neural progenitors

SOX10 has been previously described as a highly selective marker of Schwann cells^{8, 14}. This finding was in line with the key SOX10 role in neural crest development and its differentiation into glial cells and melanocytes⁶⁴. To obtain more information on the potential link between SOX10/PROM1-expressing BBC cells and Schwann cells, we inspected distribution of ROPN1, ROPN1B, MIA, GPM6B, and other genes co-expressed in BBC with either PROM1 or SOX10 across normal human cell lineages using the Primary Cell Atlas dataset (http://biogps.org/dataset/BDS_00013/). Remarkably, in addition to SOX10 and 4 genes listed above, this analysis identified six more genes as selectively expressed in Schwann cells and BBC (FIG. 3 and Suppl. FIG. 3). Of note, MIA, S100B, FABP7, and SHC4/RaLP have been previously linked to melanoma^{65–68} and glioma^{69–71}. Overall, we identified 10 basic cell lineage markers shared between ACC, BBC, melanoma, and Schwann cells that strongly supported our hypothesis on the origin of these three cancers from Schwann cell precursors (FIG. 3). Based on these associations, it is reasonable to propose that all these novel genes/markers, along with SOX10, are CSC-intrinsic factors that play important roles in aggressive cancer behavior and may be used for tracking, isolation, and selective elimination of CSC as well as for diagnostics, and prognostication of SOX10-positive cancers.

The PROM1/SOX10 gene signature is partially maintained in PDX models of BBC

Patient-derived xenografts (PDX) are becoming common tools in preclinical studies as they maintain tumor heterogeneity, support CSC, and faithfully mimic tumor response to therapy⁷². To determine if PDX models of BBC can be used for tracking and isolation of SOX10/PROM1-expressing cells, we asked if a publicly available TNBC PDX dataset, E-GEOD-47079, N=7, maintains expression of genes shown in FIG.1. This comparison identified in the TNBC dataset 56% (23/41) of the clinical PROM1 gene signature as close PROM1 correlates ($r>0.65$) suggesting that expression of at least half of the PROM1 signature genes is maintained in PDX models (data not shown). Overall, this analysis supported these models as adequate tools for CSC isolation, but also underlined differences in gene expression, which are most likely caused by model limitations, such as murine tissue environment and lack of interaction with immune system.

DISCUSSION

CSC have taken center stage in clinical research as they are considered the root of aggressive tumor behavior and resistance to cytotoxic therapies. However, identification of true CSC is challenging as CD24, CD44, CD133, and other cell surface markers used for their detection are not cell lineage- or cancer-specific. Thus, inclusion of more definitive markers for reliable and clinically relevant CSC identification is required^{4, 5}.

Molecular profiling separated clinical breast cancer specimens into four major types: luminal, ERB2-positive, basal-like (BBC), and normal breast⁷³. BBC, which comprises

~15–20% of all breast cancers, is a molecularly and clinically distinct cancer type distinguished with a myoepithelial cell-like expression profile, poor prognosis, and resistance to cytotoxic and hormone receptor-targeting therapies⁷⁴. We recently demonstrated that SOX10, a master regulator of neural stem cells and highly specific marker of Schwann cells and melanocytes, is overexpressed in clinical ACC and BBC specimens as a part of conserved gene signature. Furthermore, we identified substantial overlaps between the SOX10 gene signatures in BBC, ACC, and neuroectodermal cancers (melanoma, neuroblastoma, and glioma) suggesting that SOX10 and its gene signature demarcate CSC with common properties in cancers with similar cell origin^{14, 75}. Subsequently, we focused on ACC to demonstrate that SOX10+ cells purified from ACC tissue express CD133, a neural and cancer stem cell surface marker^{76–79}, and activate signaling pathways and genes characteristic for NSC, such as NOTCH1, FABP7, etc^{19, 75}. This novel SOX10+/CD133+ CSC population, which we named ACC-CSC, possessed stem cell properties, such as the ability to form spheroids in culture and initiate tumors with ACC histology in nude mice¹⁹. We then asked if tracking SOX10 and CD133 expression in clinical breast cancer specimens may similarly provide information on CSC in BBC.

In this study, using publicly available clinical data, we were able to track genes co-expressed with PROM1/CD133 and SOX10 across different breast cancer specimens and datasets. Moreover, we identified based on this straightforward approach a conserved gene signature highly enriched with novel and previously reported stem cell markers. Overall, gene co-expression data strongly suggested the existence in BBC of a previously unknown population of CSC cells with molecular traits similar to NSC and cancers of neural crest origin. The validity of our conclusion is strengthened by 1) successful application of similar approach to tracking and isolation of CSC from ACC; 2) cross-validation of breast cancer MEM data on an independent dataset using a different platform and calculation algorithm; 3) overlap found between our data and previously published BBC stem cell markers; and 4) identification of cell markers shared between BBC, ACC, melanoma, and Schwann cells. As we also demonstrate, PDX models produced from clinical BBC specimens further support the existence of SOX10+/CD133+ cells. It can be argued, however, that gene expression patterns derived from mixed tumor specimens by themselves cannot define cancer stem cells or any cellular subpopulations, and direct isolation of such cells from grafted or fresh tumor tissue is required. While this is true and we already initiated such experiments, it is important to note that our analysis of available BBC cell lines for SOX10 expression demonstrated that HCC1569, HCC38, and MX-1 retain expression of SOX10 and its signature elements¹⁴ supporting the existence of cells with suggested core phenotype. MX-1, one of the most aggressive triple-negative breast cancer cell lines, was especially informative as it consisted entirely of SOX10+/CD133+ cells that also expressed GABRP, FABP7, VTCN1, activated NOTCH1, and many other genes listed in FIG. 1 (our unpublished data). Thus, experiments with MX-1, one of the oldest cancer cell line/xenograft model which is available from NCI-Frederick (<https://frederick.cancer.gov/services/tumorcatalog.aspx>), may provide an important insight into molecular signaling of SOX10+/CD133+ cells related to poor prognosis and help with designing CSC-targeting therapies.

In summary, our study provides a blueprint for BBC studies focused on CSC isolation, modeling, and targeting as well as for validation of already available and newly derived cell

lines and PDX as adequate tools for CSC research. The results of our analysis are consistent with the existence in BBC of SOX10+/CD133+ cells with NSC properties and major role proposed for SOX10 in breast stem cell maintenance¹⁷. Tracking CSC in BBC using novel markers that we identified offers new research tools and approaches to improve BBD diagnostics, prognostication, and treatment.

CONCLUSION

Our study suggests that most of clinical BBC specimens contain a population of SOX10+/CD133+ CSC that coordinately express genes and markers characteristic for NSC. Highly conserved gene expression patterns associated with SOX10 and CD133/PROM1 expression suggest that these CSC can be tracked in clinical tumor specimens and PDX models via a large set of coordinately expressed NSC markers. Overall, the results of our study provide a new approach for identification and isolation of true CSC for basic research as well as diagnostics, prognostication, and pre-clinical studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of abbreviations

ACC	adenoid cystic salivary carcinoma
BBC	basal-like breast carcinoma
TNBC	triple-negative breast cancer
CSC	cancer stem cells
NSC	neural stem cells
PDX	patient-derived xenografts
RPPA	reverse phase protein array

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CLINICAL PRACTICE POINTS

While CSC eradication may be an effective solution for treating cytotoxic therapy-resistant cancers, tracking CSC in clinical specimens and their isolation from tumor tissue and patient-derived xenografts is impossible without reliable CSC markers. Our approach to CSC identification in clinical specimens is based on analysis of massive gene expression data with the goal to track potential CSC by their conserved gene signatures. We recently demonstrated that SOX10, a marker of neural crest stem cells and their derivatives, is overexpressed as a part of conserved gene signature in two histologically similar cancers resistant to radio- and chemotherapies, salivary adenoid cystic carcinoma (ACC) and basal-like breast carcinoma (BBC). Consequently, we showed that SOX10 is a reliable and specific CSC marker in ACC as it marks a novel population of tumor-initiating cells with the SOX10+/CD133+ phenotype. Here, we test our hypothesis that co-expression of these two markers may help to identify a similar population of CSC in BBC. Our results produced on clinical breast cancer gene expression datasets strongly support our hypothesis that BBC, similar to ACC and other SOX10-expressing cancers, is driven by a population of SOX10+/CD133+ cells with neural stem properties that have not been previously recognized in breast cancer. Our study suggests that SOX10+/CD133+ cells can be tracked via a large group of coordinately expressed markers in clinical tumor specimens and PDX models validating PDX models as adequate tools for CSC research and pre-clinical studies aimed at CSC eradication.

Figure 1. Top 44 genes ranked by their co-expression with PROM1 in Affymetrix U133 Plus 2.0 datasets using aggregation MEM meta-analysis

Novel genes that we associated with BBC and SOX10 are shown in red font, while previously published BBC-intrinsic genes are in blue. All genes show highly statistically significant co-expression with PROM1 and each other (p values shown at the right edge of matrix). Some genes are represented by 2 or 3 probes. Dataset IDs and their sizes (in brackets) are shown on top. Dark brown color signifies highest ranks of association by co-expression. More information on how to read MEM output can be found at <http://biit.cs.ut.ee/mem/help/help.html>.

Figure 2. Heat map of the E-GEOD-21653 dataset produced by MEM on clinical specimens sorted into 5 breast cancer subtypes illustrates BBC-selective expression of top 44 genes ranked by co-expression with PROM1 (red – high expression, green –low expression levels)
Genes whose expression is strongly selective to cancer specimens are shown with arrows.

Figure 3. Candidate CSC markers shared between ACC, BBC, melanoma, and Schwann cells
 Expression of 10 Schwann cell markers/genes is conserved in three cancers suggesting that they all originate from neural crest.

Table 1

Statistically significant associations between expression of SOX10/PROM1 signature genes in the Breast Invasive Carcinoma TCGA dataset.

Gene A	Gene B	p-Value	Log Odds Ratio
SOX10	PROM1	2.34E-13	2.22
SOX10	GPM6B	3.79E-21	3.00
SOX10	ROPN1	2.37E-42	4.02
SOX10	ROPN1B	3.72E-42	4.14
SOX10	TRIM2	1.56E-26	3.20
PROM1	GPM6B	1.06E-09	2.01
PROM1	ROPN1	7.60E-23	2.84
PROM1	ROPN1B	3.45E-21	2.83
PROM1	TRIM2	8.63E-12	2.11
GPM6B	ROPN1	9.64E-21	2.95
GPM6B	ROPN1B	8.29E-20	2.97
GPM6B	TRIM2	6.61E-23	3.13
ROPN1	ROPN1B	1.14E-70	5.81
ROPN1	TRIM2	2.83E-33	3.56
ROPN1B	TRIM2	5.21E-36	3.82