RESEARCH ARTICLE

Dissecting the Origin of Breast Cancer Subtype Stem Cell and the Potential Mechanism of Malignant Transformation

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Abstract

Background

Breast cancer is the most common incident form of cancer in women including different subtypes. Cancer stem cells (CSCs) have been confirmed to exist in breast cancer. But the research on the origin of breast cancer subtype stem cells (BCSSCs) is still inadequate.

Methods

We identified the putative origin cells of BCSSCs through comparing gene signatures between BCSSCs and normal mammary cells from multiple perspectives: common signature, expression consistency, functional similarity and shortest path length. First, the potential origin cells were ranked according to these measures separately. Then Q statistic was employed to combine all rank lists into a unique list for each subtype, to prioritize the origin cells for each BCSSC. Next, we identified origin-related gene modules through integrating functional interaction network with differentially expressed genes. Finally, transcription factors of significant gene modules were predicted by Match™.

Results

The results showed that Luminal A CSC was most relevant to luminal progenitor cell or mature luminal cell; luminal B and HER2 CSC were most relevant to bipotent-enriched progenitor cell; basal-like CSC was most relevant to bipotent-enriched progenitor cell or mature luminal cell. Network modules analysis revealed genes related to mitochondrial respiratory chain (MRC) were significantly dysregulated during the origin of luminal B CSC. In addition, SOX10 emerged as a key regulator of MRC.
Conclusions
Our study supports substantive evidence for the possible origin of four kinds of BCSSCs. Dysfunction of MRC may contribute to the origin of luminal B CSC. These findings may have important implications to treat and prevent breast cancer.

Introduction
Cancer stem cells (CSCs) are a small subpopulation of cells inside tumors. They possess the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise tumors [1]. In the latest studies, the existence of CSCs has been validated in various kinds of cancer, such as colorectal cancer, bladder cancer and breast cancer [2–4]. Moreover they are responsible for tumorigenesis, recurrence, and metastasis [5]. Currently, cancer chemotherapy is cytotoxic to the bulk of tumor cells but fails to eliminate CSCs, thereby making them the leading reason for recurrence [6]. So, eradication of CSCs is the prerequisite of cancer therapy [7].

The origin of the CSC remains elusive. There are three hypotheses [8]: 1) CSCs derived from normal adult stem cells. Adult stem cells are long-lived cells with a high proliferative capacity tending to accumulate the mutations that lead to carcinogenesis [9]. 2) CSCs derived from progenitor cells. According to this hypothesis, through the mutation occurred in progenitor cells during the process of differentiation, they can obtain the ability of self-renewal, and then form cancer stem cells [10]. 3) CSCs derived from differentiated cells. For example, a combination of epidermal growth factor receptor (EGFR) pathway activation and loss of INK4A tumor-suppressor function induces a high-grade glioblastoma multiforme phenotype from differentiated astrocytes [11]. Different types of CSC may derive from different origin and lead to the formation of heterogeneous tumor. Lottaz et al. identified two putative founder cell populations for two subtypes of glioblastoma CSCs: fetal neural stem cell and adult neural stem cell [12].

Breast cancer is the most common incident form of cancer in women worldwide and responsible for 1.4 million new cases annually [13]. Perou et al. classified breast cancers into 5 subtypes: luminal A, luminal B, HER2/Neu, basal-like and normal-like [14]. Each subtype has heterogeneous pathologies and clinical outcomes, suggesting the possibility that different subtypes of breast cancers may be derived from distinct breast cancer subtype stem cells (BCSSCs). However, it remains unclear whether different BCSSCs derived from different cells of origin. It will count for much to design personalized therapy if this key issue is clearly elucidated.

In this study, we compared the gene signatures of BCSSCs and normal mammary cells to identify the possible cellular origin of BCSSCs. Next we focused on the origin mechanism of luminal B CSC to further analyzed expression data of luminal B CSC and bipotent-enriched progenitor cell through a network module-based method in protein function interaction (FI) network. The results showed that genes related to the function of mitochondrial respiratory chain (MRC) were significantly dysregulated between normal and cancer condition. We also found SOX10 was the key regulator of genes associated with respiratory chain by transcription factor binding site analysis. Based on our data, we proposed a possible case of breast cancer subtype tumorigenesis that might explain the heterogeneity of breast cancer. These findings might have important implications for strategies to treat and prevent breast cancer.

Methods
We presented a computational pipeline to investigate the origin of BCSSCs through comparing the gene signatures of BCSSCs and normal mammary cells. We further analyzed the
mechanism of malignant transformation from normal cells to BCSSCs through network module-based method in protein FI network. The workflow of our approach is shown in Fig 1.

Preprocessing of gene expression profiles

The raw data (CEL files) of human breast cancer stem cells and normal mammary cell was downloaded from Gene Expression Omnibus (GEO) database. In this study breast cancer stem cells (GSE7513, GSE7515) [4] are classified into 4 subtypes using immunohistochemical results of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER2)[15, 16]: luminal A subtype (ER/PR+, HER2-), luminal B subtype (ER/PR+, HER2+), basal-like subtype (ER-/PR-/HER2-) and HER2 subtype (ER-/PR-/HER2+). HER2 subtype was only compiled in GSE7515. All breast cancer stem cells include CSCs and non-CSCs expression profiles. Mature mammary epithelial cells are generated from progenitor cells and progenitor cells are generated from primitive bipotent cells through a hierarchical process. GSE11395[17] subdivide normal human mammary epithelial cells by surface markers including the expression profiles of bipotent-enriched progenitor cells (MUC1\(\text{CD133}^+\text{CD10/THY1}^+\text{CD49f}^+\)) and committed luminal progenitor cells (MUC1\(\text{CD133}^+\text{CD10/THY1}^+\text{CD49f}^+) as well as mature luminal ((MUC1/\text{CD133}^+\text{CD10/THY1}^+\text{CD49f}^+) and myoepithelial cells (MUC1\(\text{CD133}^+\text{CD10/THY1}^+\text{CD49f}^+\)).

We normalized gene expression arrays using robust multiarray analysis (RMA) methods as implemented in the software BRB ArrayTools [18]. Based on log2-transformed data, two-sided t test was used to identify significant difference of gene expression between groups of samples. Meanwhile, we computed fold changes by comparing the mean gene expression levels between groups. In BCSSCs respect, we compared gene expression between each kind of BCSSC and corresponding non-CSC. We defined genes with significant p value (<0.05) and > 2 fold-change as up-regulated signature genes of each kind of BCSSC. On the other hand, genes with significant p value and < 0.5 fold-change were defined as down-regulated signature genes. In normal cells respect, we compared gene expression between each population and others. We defined genes with significant p value and > 2 fold-change as over-expressed signature genes of each kind of normal population. On the other hand, genes with significant p value and < 0.5 fold-change were defined as under-expressed signature genes.

Enrichment analysis of signatures

The statistical significance of the overlaps between each kind of BCSSC and normal cell signature gene sets was calculated using the hypergeometric distribution. The \(P\) value of enrichment is calculated by

\[
P(x \geq q) = 1 - \sum_{x=1}^{q-1} \binom{n}{x} \binom{N-n}{M-x},
\]

where \(n\) is the total number of genes, \(M\) and \(N\) is the number of signature gene sets of BCSSCs and normal cells, respectively, and \(x\) is the number of genes in common. We conducted this analysis for each pair of normal over-expressed and BCSSC up-regulated signature genes as well as each pair of normal under-expressed and BCSSC down-regulated signature genes, respectively. Two cancer stem cell profiles were analyzed separately.
Gene signature score (GSS)
For each normal mammary sample, a GSS was computed to measure the concordance of expression with each BCSSC. Higher score indicates the normal breast sample is more inclined to acquire the gene signature of the given BCSSC. GSS is defined as follow:

\[
GSS = \sum \frac{x_g \cdot (y_g - z_g)}{\sum x_g},
\]

where the sum is up-regulated or down-regulated genes in each BCSSC signature gene sets, \(x_g\) is the log2-fold-change of expression value for that gene in the BCSSC signature; \(y_g\) is log2-expression value for the same gene in one normal breast sample and \(z_g\) is log2-mean expression value of the same gene in all normal breast samples. We computed GSS for two cancer stem cell profiles separately.

Semantic similarity score (SSS)
We evaluated the functional similarity between signatures of normal cell and BCSSC by gene ontology (GO) term's semantic similarity. We calculated the SSS for each pair of normal over-expressed and BCSSC up-regulated signature genes as well as each pair of normal under-expressed and BCSSC down-regulated signature genes. We performed this analysis for two cancer stem cell profiles separately. This process was implemented by R package "GOSemSim" [19], which was developed to compute semantic similarity among GO terms, sets of GO terms, gene products and gene clusters based on the graph structure of GO. Here, we only used biological process category in GO, and selected "Lin" and "BMA" methods in "GOSemSim" to compute the similarity scores.

Shortest path (SP) length
In protein-protein interaction network, Human Protein Reference Database (HPRD), we calculated the average length of the SP length between all possible pairs of nodes for normal over-expressed and BCSSC up-regulated signature genes or normal under-expressed and BCSSC down-regulated signature genes by R package "igraph". We performed this analysis for two cancer stem cell profiles separately.

Integration of the rank lists
We detected the relationship between signatures of normal cell and BCSSC from four different aspects including enrichment analysis, BCSSC gene signature score, GO term's semantic similarity and shortest path for up-regulated/over-expressed and down-regulated/under-expressed signature genes. Consequently, we obtained eight rank lists of putative origins for each kind of BCSSCs according to these four values: \(p\)-value of hypergeometric distribution, GSS, SSS and
the length of SP. In order to get an overall rank list for all pairs of normal cells and BCSSCs, we fused the eight separate rank lists using a \( Q \) statistic [20, 21]. The formula was as follows:

\[
Q(r_1, r_2, \ldots, r_N) = N!V_N
\]

\[
V_k = \sum_{i=1}^{k} (-1)^{i-1} \frac{V_{k-i}}{i} \frac{N!}{(N-k+1)!}
\]

\[
V_0 = 1,
\]

where \( N \) representing number of separate rank lists used, and \( r_i \) is the rank ratio for the \( i \)-th rank list. Variable \( V \) is an intermediate variable.

Identification of origin-specific gene modules

In order to further investigate the meaningful and biologically relevant subunits, we explored "origin-specific modules". Here, we identified modules whose genes were both topologically close in network and highly correlated in expression level by a cytoscape plug-in, Reactome FI [22]. The method first calculated the Pearson correlation coefficients (PCCs) among all FI pairs in the gene expression data set, and then assigned the PCCs to the edges of the FI network. Next, it used a highly efficient network clustering algorithm to cluster the weighted network into a series of gene interaction modules. We applied this approach to gene expression data of luminal B CSC and bipotent-enriched progenitor cell, respectively.

For the modules obtained we firstly filtered the modules passed to the next step of analysis by removing whose number of nodes was smaller than default threshold, and which had an average PCC below 0.5. Second, for the filtered list of modules, we mapped "origin-specific signature" genes to find out significantly enriched modules (hypergeometric distribution test \( p < 0.05 \)). Next, we investigated overlapped genes between CSC and normal cells to find modules that shared common "origin-specific signature" genes.

Prediction of transcription factor

In order to study the upstream regulatory elements, we conducted prediction of transcription factor by Match\(^\text{TM}\) that used the matrix library collected in TRANSFAC [23]. We obtained transcripts of genes from UCSC. 2000bp promoter upstream sequence of each transcript was extracted as the transcription factor binding regions. We applied minimize false positive rate (minFP) option and set core similarity cut-off to 1 to acquire the strictest output.

Results

Identification of gene signatures in normal mammary cells or BCSSCs

To gain insight into the molecular characteristics of normal and malignant breast cells, we downloaded gene expression profiles of normal mammary cells and BCSSCs data from GEO. In normal mammary cells comparison (one population vs. others), 138, 218, 66 and 333 genes were significantly under-expressed in bipotent progenitor cells, committed luminal progenitor cells, mature luminal and myoepithelial cells, respectively. 307, 283, 314 and 318 genes were significantly over-expressed in bipotent progenitor cells, committed luminal progenitor cells, mature luminal and myoepithelial cells, respectively (\( t \)-test \( p < 0.05 \) and two-fold differentially expressed, Table 1, details in methods section). We defined the significantly differentially expressed genes of each kind of normal cells as signature gene sets, including under- and over-expressed genes. In BCSSCs comparison, we compared gene expression in two BCSSCs
expression profiles separately for each subtype (CSCs vs. non-CSCs) using the same criteria. In the respect of up-regulated genes, we identified 361, 270 and 286 genes in GSE7513 for luminal A, luminal B, basal-like CSCs respectively and 218, 379, 875 and 448 genes in GSE7515 for luminal A, luminal B, basal-like and HER2 CSCs respectively. In the respect of down-regulated genes, we identified 188, 890 and 129 genes in GSE7513 for luminal A, luminal B, basal-like CSCs respectively and 199, 1251, 931 and 1115 genes in GSE7515 for luminal A, luminal B, basal-like and HER2 CSCs respectively (Table 2, details in methods section). Up-regulated and down-regulated genes together were the signature gene sets of BCSSCs. So far we defined signature gene sets for each kind of normal mammary cell and BCSSC.

Detection of BCSSC origin through integrating the characteristic of signatures

We investigated the correlation between the signatures of each kind of normal mammary cell and the signatures of each kind of BCSSC in two expression profiles. First, hypergeometric distribution was employed to conduct enrichment analysis. We compared BCSSC up-regulated with normal cell over-expressed signature genes and BCSSC down-regulated with normal cell under-expressed signature genes separately (S1A Fig). Lower p-value represented that two gene sets shared more signature genes. Next, we computed the GSS of BCSSC for each normal cell population to further explore the correlation on gene expression level (details in methods section, S1B Fig). We also calculated the GSS using up-regulated and down-regulated BCSSC signature genes separately. The higher GSS indicated the up-regulated BCSSC signature genes tended to be more highly expressed in a given normal cell, or the down-regulated BCSSC signature genes were more lowly expressed in this normal cell.

Next, we evaluated the functional similarity of signatures genes. GO-term's semantic similarity has been proposed for comparing genes at the functional level on the basis of GO annotation [24]. A higher value of SSS indicated a greater functional similarity between signatures of BCSSC and normal cells (details in methods section, S1C Fig).

Finally, we compared signatures on the network topological structure level. The shortest paths algorithm has been applied to discover novel candidate disease related genes through known disease related genes in the protein-protein interaction network [25, 26]. The SP between two nodes was the smallest distance value in their corresponding paths [27]. A smaller

| cell population          | over-expressed | under-expressed |
|--------------------------|----------------|-----------------|
| bipotent progenitor cells| 307            | 138             |
| luminal progenitor cells | 283            | 218             |
| mature luminal cells     | 314            | 66              |
| myoepithelial cells      | 318            | 333             |

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value of SP indicated a stronger relevance between signatures of BCSSC and normal cells (details in methods section, S1D Fig).

We ranked the eight values \((p\)-values of hypergeometric distribution, GSS, SSS and SP for up-regulated/over-expressed and down-regulated/under-expressed signature genes) for each kind of breast cancer subtype and four normal mammary cells. We integrated the eight rank lists for each breast cancer expression profiles by \(Q\) statistic [21] and implemented by Endeavour program [20]. Two rank lists were obtained for each breast cancer subtype: one for GSE7513, another for GSE7515. HER2 subtype only had one rank order because it was only compiled in GSE7515. The average of these two rank orders was called the overall rank. Top of the final list was the possible cellular origin for each kind of BCSSC. The results showed that luminal A CSC was most relevant to luminal progenitor cell or mature luminal cell; luminal B and HER2 CSC were most relevant to bipotent-enriched progenitor cell; basal-like CSC was most relevant to bipotent-enriched progenitor cell or mature luminal cell (Fig 2). The luminal A subtype is ER-positive and the least aggressive breast cancer molecular subtype [28] and might arise through ER-positive progenitors such as luminal progenitor cells [29]. Some researchers indicated that luminal B tumors might derive from ER-negative progenitor cell, which could undergo luminal lineage differentiation and display a positive expression of ER [30]. In addition, luminal B, basal-like and HER2 subtypes all had the possibility to origin from bipotent-enriched progenitor cell as the result showed. It’s credible because there existed common genomic alterations in basal-like, HER2 and luminal B breast tumors suggesting a common cell origin of these tumor subtypes [29].
Functional analysis of differentially expressed genes between luminal B CSC and bipotent-enriched progenitor cell

Basal-like and HER2-enriched subtypes displayed the worst prognosis followed by luminal B, whereas luminal A has a good prognosis [31]. However, luminal B tumors often have the worst long-term prognosis, as exhibiting rapid recurrence after endocrine therapy compared to basal-like and HER2-enriched tumors [32]. Thus, we focused on this origin of luminal B subtype in the following analysis. In order to investigate the potential mechanism of malignant transformation, we detected the difference in gene expression patterns between luminal B CSC and bipotent-enriched progenitor cell (luminal B CSC vs. bipotent-enriched progenitor cell). 6033 overlapped differentially expressed genes (1999 up-regulated and 4034 down-regulated) between GSE7513 and GSE7515 were detected (the same criteria as above, Fig 3A) termed “origin-specific signature”.

We next conducted functional enrichment analysis of the entire “origin-specific signature” genes based on GO [33]. In order to reduce the redundancy to find more statistically and biologically meaningful function, we chose R package “GOFuntion” to implement the analysis [34]. The majority of significantly enriched biological process terms included cell cycle, response to DNA damage stimulus, signal transduction by p53 class mediator and DNA replication, which reflected known properties of stem cells and breast cancer (Fig 3B). It was interesting to note that substantial portions of cellular component terms were associated with mitochondrion. In addition, MRC was also one of significantly enriched terms of biological process and molecular function. This phenomenon arrested our attention. We discovered that numerous studies have suggested that mitochondrial processes might play important roles in tumor initiation and progression. Mitochondrial dysfunction clones of breast cancer cells exhibited higher migration and invasive behaviors compared with their parental cells [35]. Increased incidence of mtDNA polymorphisms were detected in breast cancer samples compared with normal samples [36]. These results indicated that MRC function may play a key role in promoting transformation from bipotent-enriched progenitor cell to luminal B CSC. We should focus on it in following study.

Identification of origin-specific gene modules

In order to further investigate the meaningful and biologically relevant subunits, we explored “origin-specific modules”, that is, dysfunction of these modules might cause malignant transformation. Topological properties of protein FI network have been studied to extract new disease-related knowledge [37], and co-expressed genes of protein-protein interaction network are highly connected indicating that they are functionally related [38]. Here, we identified modules whose genes were both topologically close in network and highly correlated in expression level by a cytoscape plug-in, Reactome FI. We applied this approach to gene expression data of luminal B CSC and bipotent-enriched progenitor cell, respectively.

Two modules for each condition were obtained after filtration (details in methods section). Module 1 from luminal B CSC and module 3 from bipotent-enriched progenitor cell shared common “origin-specific signature” genes (Fig 4A, S1 and S2 Tables); module 2 from luminal B CSC and module 4 from bipotent-enriched progenitor cell shared common “origin-specific signature” genes (S2 Fig).

We performed functional enrichment analysis for four modules by DAVID. An amazing discovery for us was that module 1 and module 3 were significantly enriched in MRC function on all three GO categories ($p<0.05$, Fig 4B and S3 Fig). Basically all terms associated with MRC including electron transport chain, cellular respiration and ATP synthesis coupled electron
transport were enriched. It is incredibly impressive that this result showed astonishing consistency with what we detected above.

The common "origin-specific signature" genes shared by module 1 and 3 were virtually all down-regulated in luminal B CSC except NDUFA11 (Fig 4C). It have been previously described that a low amount of MRC complexes was well adapted to the low energy demand of this G0-G1 resting stem cell type [39]. Li LD, et al further discovered that NDUFA9 played a pivotal role in stem cell self-renewal and cancer growth because it uniquely expressed in human embryonal carcinoma cells comparing with human embryonic stem cells [40].

Fig 3. Differentially expressed genes between luminal B CSC and bipotent-enriched progenitor cell. A, heat map of differentially expressed genes between luminal B CSC and bipotent-enriched progenitor cell. Each row represents a gene; each column, a sample. Colored bar indicates the expression level. B, the result of functional enrichment analysis of differentially expressed genes based on GO. The terms in red are related to mitochondrion. The disconnected bar represents p-value of the term is zero.

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Moreover, NDUFB9 was down-regulated in highly metastatic breast cancer cells compared with parental breast cancer cell line which indicating that NDUFB9 promoted breast cancer cells proliferation, migration and invasion [41]. On the other hand, inefficient MRC function has been implicated in the vicious cycle of reactive oxygen species (ROS) production which increased risk of breast cancer [42]. Moreover, human mammary carcinoma cells were shown to have depressed expression levels of complex I (NADH-ubiquinone oxidoreductase) genes, the transfer of electrons from NADH to respiratory chain [43]. These previous discoveries confirmed our results. The gene and complex involvement of MRC function in stem cell and breast cancer implied its pivotal role in the process of the origination of luminal B CSC. So we defined module 1 of cancer state as “origin-specific module”.

**Transcriptional regulation of origin-specific module**

In order to study the upstream regulatory elements, we conducted prediction of transcription factor for module 1 by Match™. We obtained nine transcripts for nine genes of module 1. As a result, we found the transcription factor binding site of SOX10 existed in the promoters of seven transcripts in module 1 (Fig 4D). SOX10 was validated as a sensitive diagnostic marker for breast cancer [44] as well as a marker and principal regulator of neural crest stem cells (NCSCs) playing an important role in the maintenance and migration of NCSCs [45]. In
addition, it was also to be confirmed that SOX10 is peripherally associated with the mitochondrial outer membrane and raise the possibility of signal transduction cascade between the nucleus and mitochondria [46]. In conclusion, SOX10 is associated with breast cancer, stem cell and mitochondria function. Our findings corroborated these discoveries from another point of view suggesting dysregulation of SOX10 might facilitate dysfunction of MRC, thus prompting malignant transformation of bipotent-enriched progenitor cell.

Discussion

The identification and comprehension of breast CSCs is rapidly progressing recent years. Some have postulated that eradication of CSCs is the prerequisite of cure for cancer because they are responsible for tumorigenesis, recurrence, and metastasis [5]. But the fundamental question that which kind of normal cell is the key cellular target for malignant transformation is still unknown. Here, this present work attempted to provide a broad view of the origin of CSC of distinct breast cancer subtypes through a high throughput manner.

We compare the signatures between BCSSCs and their putative normal cells of origin from different aspects including enrichment analysis, BCSSC gene expression score, GO-term’s semantic similarity and the shortest path in HPRD network for up-regulated/over-expressed and down-regulated/under-expressed signature genes. We finally obtained eight distinct rank lists for each BCSSC gene expression profile. Through integrating the rank lists, we found the relationships associated with bipotent-enriched progenitor cell ranked frequently at the top of the list. This result is reasonable considering the known characteristic of progenitor cell. Bipotent-enriched progenitor cell was long-lived cells possessing pluripotency. Mutations in tumor suppressor genes or oncogenes were incidental which might affect differentiation potential of progenitor cells that drove tumor phenotypes [47].

The relationship of luminal B CSC and bipotent-enriched progenitor cell ranked higher than other origin pairs. In fact, the overlapped genes between luminal B CSC and bipotent-enriched progenitor cell signature indeed had stem cell related function. For example, ST7 and USP11, two of the overlapped up-regulated/over-expressed genes between luminal B CSC and bipotent-enriched progenitor cell, existed in gene set that represented the core expression signature of embryonic stem (ES) cells. Function of this gene set reflected the activity of the regulatory pathways associated with their stem-like character [48]. Furthermore, five down-regulated/under-expressed overlapped genes (EPS8, IRX5, MYO5B, GUCY1A3 and TNFRSF1B) were in the gene set representing under-expressed genes bounding by Polycomb Repressive Complex 2 in human ES cells [49].

The “origin-specific module” we found through network modules analysis was significantly associated with MRC function. Mitochondrion is a critical organelle in eukaryocyte. It has been established that mitochondria burn the calories in our diet with the oxygen to make energy and heat to maintain our body temperature through ATP synthesis. All enzymes and coenzymes involved in this process constitute MRC. As a by-product of energy production, the mitochondria also generate the endogenous reactive oxygen species (ROS) which is crucial not only in redox signaling for many cellular events, but also to be pivotal in variety of harmful oxidative damage under pathological conditions [50, 51]. The low gene expression of MRC complexes is requisite for existence of stem cell type [39]. But in the meantime, it also produces more ROS production which increased risk of breast cancer [42]. It is accord with our result that genes of MRC are down-regulated in luminal B CSC. The phenomenon that lower expression value of these genes in luminal B CSC compared with progenitor cell maybe implies that CSCs are less differentiated cells, which means their growth pattern and tendency to spread is more reckless. Finally, transcription factors such as SOX10 which directly targeted...
mitochondria-associated genes may represent new targets for the elimination or modulation of luminal B CSC.

There are also some other studies of possible mechanisms about origin of luminal B CSC. Previous research revealed that histone acetyltransferase KAT6A played key role in maintaining the ability of self-renewal and activity of luminal breast cancer stem cell [52]. Bone morphogenetic protein (BMP) pathway and Wnt pathway was also reported to be the prominent mechanisms of quiescence and acquisition of stemness in luminal breast cancer cell lines [53]. The association between these possible mechanisms should be further discussed to ascertain the specific mechanism of origin of CSC.

**Conclusion**

The data of our study support substantive evidence for the possible origin of four kinds of BCSSCs and the mechanism of malignant transition from bipotent-enriched progenitor cells to luminal B CSCs. These discoveries is striking, since, in spite of dysfunction of MRC has been confirmed during cancer development, the research disclosed its key role in origin of cancer stem cell is absent. But further expression profile studies will be necessary to further determine the origin of breast tumor phenotypes. Currently limited gene expression data of breast cancer stem cell was the biggest restrictions of our study. Our discoveries also need to be confirmed by low-throughput experiments. In the longer term, detailed characterization of the modules active or repressive in CSCs is likely to yield powerful diagnostic and prognostic markers and, quite possibly, the upstream regulatory elements will be the attractive targets for eliminating CSCs to prevent recurrence and improve survival in breast cancer patients.

**Supporting Information**

**S1 Fig.** Comparing gene signatures of normal mammary cells and BCSSCs. A, the result of hypergeometric distribution. B, BCSSC gene signature scores (GSS) for each cell population in each normal sample. C, the semantic similarity score (SSS) for each pair of BCSSC and normal cell signature. D, the shortest path lengths (SP) between signatures of normal cells and BCSSCs.

**S2 Fig.** Module 2 from luminal B CSC (left) and module 4 from bipotent-enriched progenitor cell (right).

**S3 Fig.** The result of functional enrichment analysis of module 3. The functional enrichment analysis was conducted by GO. The terms in red are related to mitochondrion.

**S1 Table.** Genes in module 1.

**S2 Table.** Genes in module 3.

**Author Contributions**

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Funding acquisition: WJ.
Methodology: DML SYW.
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References
1. Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, et al. Cancer stem cells—perspectives on current status and future directions: AACR Workshop on cancer stem cells. Cancer research. 2006; 66(19):9339–44. doi: 10.1158/0008-5472.CAN-06-3126 PMID: 16990346
2. Grillet F, Bayet E, Villeronce O, Zappia L, Lagerqvist EL, Lunke S, et al. Circulating tumour cells from patients with colorectal cancer have cancer stem cell hallmarks in ex vivo culture. Gut. 2016.
3. Yang Z, Li C, Liu H, Zhang X, Cai Z, Xu L, et al. Single-cell sequencing reveals variants in ARID1A, GPRC5A and MLL2 driving self-renewal of human bladder cancer stem cells. European urology. 2016.
4. Creighton CJ, Li X, Landis M, Dixon JM, Neumeister VM, Sjolund A, et al. Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106(33):13820–5. doi: 10.1073/pnas.0907181106 PMID: 19666589
5. Dick JE. Stem cell concepts renew cancer research. Blood. 2008; 112(13):4793–807. doi: 10.1182/blood-2008-08-177941 PMID: 19064739
6. Zhou BB, Zhang H, Damelin M, Geles KG, Grindley JC, Dirks PB. Tumour-initiating cells: challenges and opportunities for anticancer drug discovery. Nature reviews Drug discovery. 2009; 8(10):806–23. doi: 10.1038/nrd2137 PMID: 19794444
7. Nguyen LV, Vanner R, Dirks P, Eaves CJ. Cancer stem cells: an evolving concept. Nature reviews Cancer. 2012; 12(2):133–43. doi: 10.1038/nrc3184 PMID: 22237392
8. Bjerkvig R, Tysnes BB, Aboody KS, Najbauer J, Terzis AJ. Opinion: the origin of the cancer stem cell: current controversies and new insights. Nature reviews Cancer. 2005; 5(11):899–904. doi: 10.1038/nrc1740 PMID: 16327766
9. Wijaya L, Agustina D, Lizardi AO, Kartawinata MM, Sandra F. Reversing breast cancer stem cell into breast somatic stem cell. Current pharmaceutical biotechnology. 2011; 12(2):189–95. PMID: 21044008
10. Molyneux G, Geyer FC, Magnay FA, McCarthy A, Kendrick H, Natrajan R, et al. BRCA1 basal-like breast cancers originate from luminal epithelial progenitors and not from basal stem cells. Cell stem cell. 2010; 7(3):403–17. doi: 10.1016/j.stem.2010.07.010 PMID: 20804975
11. Bachoo RM, Maher EA, Ligon KL, Sharpless NE, Chan SS, You MJ, et al. Epidermal growth factor receptor and InK4a/Arf: convergent mechanisms governing terminal differentiation and transformation along the neural stem cell to astrocyte axis. Cancer cell. 2002; 1(3):269–77. PMID: 12086863
12. Lotta C, Beier D, Meyer K, Kumar P, Hermann A, Schwarz J, et al. Transcriptional profiles of CD133+ and CD133− glioblastoma-derived cancer stem cell lines suggest different cells of origin. Cancer research. 2010; 70(5):2030–40. doi: 10.1158/0008-5472.CAN-09-1707 PMID: 20145155
13. Boyle P. Triple-negative breast cancer: epidemiological considerations and recommendations. Annals of oncology: official journal of the European Society for Medical Oncology / ESMO. 2012; 23 Suppl 6: vi7–12.
14. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature. 2000; 406(6797):747–52. doi: 10.1038/35021093 PMID: 10963602
15. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thurlimann B, Senn HJ. Strategies for subtypes—dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Annals of oncology: official journal of the European Society for Medical Oncology / ESMO. 2011; 22(8):1736–47.
16. Seshie B, Adu-Arvee NA, Dedey F, Calys-Tagoe B, Clegg-Lamptey JN. A retrospective analysis of breast cancer subtype based on ER/PR and HER2 status in Ghanaian patients at the Korle Bu Teaching Hospital, Ghana. BMC clinical patholgy. 2015; 15:14. doi: 10.1186/s12907-015-0014-4 PMID: 26161039
17. Raouf A, Zhao Y, To K, Stengel J, Delaney A, Barbara M, et al. Transcriptome analysis of the normal human mammary cell commitment and differentiation process. Cell stem cell. 2008; 3(1):109–18. doi: 10.1016/j.stem.2008.05.018 PMID: 18593563

18. Simon R, Lam A, Li MC, Ngan M, Menenzes S, Zhao Y. Analysis of gene expression data using BRB-ArrayTools. Cancer informatics. 2007; 3:11–7. PMID: 19455231

19. Yu G, Li F, Qin Y, Bo X, Wu Y, Wang S. GOSemSim: an R package for measuring semantic similarity among GO terms and gene products. Bioinformatics. 2010; 26(7):976–8. doi: 10.1093/bioinformatics/btq064 PMID: 20179076

20. Aerts S, Lambrechts D, Maity S, Van Loo P, Coessens B, De Smet F, et al. Gene prioritization through genomic data fusion. Nature biotechnology. 2006; 24(5):537–44. doi: 10.1038/nbt1203 PMID: 16680138

21. Stuart JM, Segal E, Koller D, Kim SK. A gene coexpression network for global discovery of conserved genetic modules. Science. 2003; 302(5643):249–55. doi: 10.1126/science.1087447 PMID: 12934013

22. Wu G, Stein L. A network module-based method for identifying cancer prognostic signatures. Genome biology. 2012; 13(12):R112. doi: 10.1186/gb-2012-13-12-r112 PMID: 23228031

23. Kel AE, Gossling E, Reuter I, Cheremushkin E, Kel-Margoulis OV, Wingender E. MATCH: A tool for searching transcription factor binding sites in DNA sequences. Nucleic acids research. 2003; 31(13):3576–9. PMID: 12824369

24. Mazandu GK, Mulder NJ. Information content-based gene ontology semantic similarity approaches: toward a unified framework theory. BioMed research international. 2013; 2013:292063. doi: 10.1155/2013/292063 PMID: 24078912

25. Zhang J, Jiang M, Yuan F, Feng KY, Cai YD, Xu X, et al. Identification of age-related macular degeneration related genes by applying shortest path algorithm in protein-protein interaction network. BioMed research international. 2013; 2013:523415. doi: 10.1155/2013/523415 PMID: 24455700

26. Li BQ, You J, Chen L, Zhang J, Zhang N, Li HP, et al. Identification of lung-cancer-related genes with the shortest path approach in a protein-protein interaction network. BioMed research international. 2013; 2013:267375. doi: 10.1155/2013/267375 PMID: 23762832

27. Strogatz SH. Exploring complex networks. Nature. 2001; 410(6825):268–76. doi: 10.1038/35065725 PMID: 11258382

28. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proceedings of the National Academy of Sciences of the United States of America. 2001; 98(19):10869–74. doi: 10.1073/pnas.191367098 PMID: 11553815

29. Davenport AJ, Ong EG, Hua J, Wei CL, Dougherty ER, et al. A novel method for ranking genes based on network structure. Bioinformatics. 2009; 25(1):111–6. doi: 10.1093/bioinformatics/btp403 PMID: 19317907

30. Melchor L, Benitez J. An integrative hypothesis about the origin and development of sporadic and familial breast cancer subtypes. Carcinogenesis. 2008; 29(8):1475–82. doi: 10.1093/carcin/bg n157 PMID: 18596026

31. Ringner M, Staaf J, Jonsson G. Nonfamilial breast cancer subtypes. Methods Mol Biol. 2013; 973:279–95. doi: 10.1007/978-1-62703-281-0_18 PMID: 23412797

32. Witkiewicz AK, Knudsen ES. Retinoblastoma tumor suppressor pathway in breast cancer: prognosis, precision medicine, and therapeutic interventions. Breast cancer research: BCR. 2014; 16(3):207. doi: 10.1186/bcr3652 PMID: 25223880

33. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nature genetics. 2000; 25(1):25–9. doi: 10.1038/75556 PMID: 10802651

34. Wang J, Zhou X, Zhu J, Gu Y, Zhao W, Zou J, et al. GO-function: deriving biologically relevant functions from statistically significant functions. Briefings in bioinformatics. 2012; 13(2):216–27. doi: 10.1093/bib/bbr041 PMID: 21705405

35. Ma J, Zhang Q, Chen S, Fang B, Yang Q, Chen C, et al. Mitochondrial dysfunction promotes breast cancer cell migration and invasion through HIF1alpha accumulation via increased production of reactive oxygen species. PloS one. 2013; 8(7):e69485. doi: 10.1371/journal.pone.0069485 PMID: 23922721

36. Czarnecka AM, Klemba A, Krawczyk T, Zdrozny M, Arnold RS, Bartnik E, et al. Mitochondrial NADH dehydrogenase polymorphisms as sporadic breast cancer risk factor. Oncology reports. 2010; 23(2):531–5. PMID: 20043118

37. Jonsson PF, Bates PA. Global topological features of cancer proteins in the human interactome. Bioinformatics. 2006; 22(18):2291–7. doi: 10.1093/bioinformatics/btl390 PMID: 16844706
38. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proceedings of the National Academy of Sciences of the United States of America. 1998; 95(25):14863–8. PMID: 9843981

39. Swartz HM, Dunn JF. Measurements of oxygen in tissues: overview and perspectives on methods. Advances in experimental medicine and biology. 2003; 530:1–12. PMID: 14562699

40. Li LD, Sun HF, Liu XX, Gao SP, Jiang HL, Hu X, et al. Down-Regulation of NDUFB9 Promotes Breast Cancer Cell Proliferation, Metastasis by Mediating Mitochondrial Metabolism. PloS one. 2015; 10(12): e0144441. doi: 10.1371/journal.pone.0144441 PMID: 26641458

41. Dormeyer W, van Hoof D, Braam SR, Heck AJ, Mummery CL, Krijgsveld J. Plasma membrane proteomics of human embryonic stem cells and human embryonal carcinoma cells. Journal of proteome research. 2008; 7(7):2936–51. doi: 10.1021/pr800056j PMID: 18489135

42. Bai RK, Leal SM, Covarrubias D, Liu A, Wong LJ. Mitochondrial genetic background modifies breast cancer risk. Cancer research. 2007; 67(10):4687–94. doi: 10.1158/0008-5472.CAN-06-3554 PMID: 17510395

43. Putignani L, Raffa S, Pescosolido R, Aimati L, Signore F, Terrisi MR, et al. Alteration of expression levels of the oxidative phosphorylation system (OXPHOS) in breast cancer cell mitochondria. Breast cancer research and treatment. 2008; 110(3):439–52. doi: 10.1007/s10549-007-9738-x PMID: 17899367

44. Ivanov SV, Panaccione A, Nonaka D, Prasad ML, Boyd KL, Brown B, et al. Diagnostic SOX10 gene signatures in salivary adenoid cystic and breast basal-like carcinomas. British journal of cancer. 2013; 109(2):444–51. doi: 10.1038/bjc.2013.326 PMID: 23799842

45. Miyahara K, Kato Y, Koga H, Dizon R, Lane GJ, Suzuki R, et al. Visualization of enteric neural crest cell migration in SOX10 transgenic mouse gut using time-lapse fluorescence imaging. Journal of pediatric surgery. 2011; 46(12):2305–8. doi: 10.1016/j.jpedsurg.2011.09.020 PMID: 22152870

46. Mou Z, Tapper AR, Gardner PD. The armadillo repeat-containing protein, ARMCX3, physically and functionally interacts with the developmental regulatory factor Sox10. The Journal of biological chemistry. 2009; 284(20):13629–40. doi: 10.1074/jbc.M901177200 PMID: 19304657

47. Proia TA, Keller PJ, Gupta PB, Klebba I, Jones AD, Sedic M, et al. Genetic predisposition directs breast cancer phenotype by dictating progenitor cell fate. Cell stem cell. 2011; 8(2):149–63. doi: 10.1016/j.stem.2010.12.007 PMID: 21295272

48. Ben-Porath I, Thomson MW, Carey VJ, Ge R, Bell GW, Regev A, et al. An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. Nature genetics. 2008; 40(5):499–507. doi: 10.1038/ng.127 PMID: 18443585

49. Lee TJ, Jennr RG, Boyer LA, Guenther MG, Levine SS, Kumar RM, et al. Control of developmental regulators by Polycomb in human embryonic stem cells. Cell. 2006; 125(2):301–13. doi: 10.1016/j.cell.2006.02.043 PMID: 16630818

50. Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annual review of genetics. 2005; 39:359–407. doi: 10.1146/annurev.genet.39.110304.095751 PMID: 16285865

51. Kagawa Y, Cha SH, Hasegawa K, Hamamoto T, Endo H. Regulation of energy metabolism in human cells in aging and diabetes: FoF(1), mtDNA, UCP, and ROS. Biochemical and biophysical research communications. 1999; 266(3):662–76. doi: 10.1006/bbrc.1999.1884 PMID: 10603304

52. Turner-Ivey B, Guest ST, Irish JC, Kappler CS, Garrett-Mayer E, Wilson RC, et al. KAT6A, a chromatin modifier from the 8p11-p12 amplicon is a candidate oncogene in luminal breast cancer. Neoplasia. 2014; 16(8):644–55. doi: 10.1016/j.neo.2014.07.007 PMID: 25220592

53. Amin R, Morita-Fujimura Y, Tawarayama H, Semb a K, Chiba N, Fukumoto M, et al. DeltaNp63alpha induces quiescence and downregulates the BRCA1 pathway in estrogen receptor-positive luminal breast cancer cell line MCF7 but not in other breast cancer cell lines. Molecular oncology. 2016; 10(4):575–93. doi: 10.1016/j.molonc.2015.11.009 PMID: 26704768