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Current Update of Patient-Derived Xenograft Model for Translational Breast Cancer Research

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Abstract

Despite recent advances in the treatment of patients with breast cancer (BrCa), BrCa remains the third leading cause of cancer death for women in the US due to intrinsic or acquired resistance to therapy. Continued understanding of gene expression profiling and genomic sequencing has clarified underlying intratumoral molecular heterogeneity. Recently, the patient-derived xenograft (PDX) models have emerged as a novel tool to address the issues of BrCa genomics and tumor heterogeneity, and to critically transform translational BrCa research in the preclinical setting. PDX models are generated by xenografting cancer tissue fragments obtained from patients to immune deficient mice, and can be passaged into next generations of mice. Generally, in contrast to conventional xenograft using cancer cell lines, PDXs are biologically more stable and recapitulate the individual tumor morphology, gene expression, and drug susceptibility of each patient. PDX may better model the original patient's tumor by retaining tumor heterogeneity, gene expression, and similar response to treatment. PDX models are thus thought to be more translationally relevant, especially as a drug development tool, because PDXs can capture the genetic character and heterogeneity that exists within a single patient's tumor and across a population of patients' tumors. PDX models also hold enormous potential for identifying predictive markers for therapeutic response. It has been repeatedly shown that PDX models demonstrate similar levels of activity as compared to the clinical response to therapeutic interventions. Therefore, this enables identification of therapeutic interventions that can most

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likely benefit a patient. This allows us to address the issues of BrCa genomics and tumor heterogeneity using PDXs in “pre-clinical” trials. Herein, we reviewed recent scientific development and future perspectives using PDX models in BrCa.

Keywords

Breast cancer; patient-derived xenograft model; translational research; preclinical model

Background

Since the 1950s, the overall breast cancer (BrCa) incidence in the United States (U.S.) has been gradually increasing, with stabilization of the number of new female breast cancer cases only during the last 10 years[1]. While mortality rates have been declining over recent decades, with larger decreases observed for women younger than 50-years, it is estimated that 40,450 people will die of BrCa this year[1]. The high mortality rate for BrCa is driven by poor long-term prognosis despite the emergence of new therapeutic agents for BrCa [2–5]. In order to improve BrCa prognosis, personalized treatment strategies are urgently needed that encompass tumor heterogeneity and patient diversity. Current translational research demonstrates individual diversity of intrinsic breast cancer subtypes based on genome wide analysis [6–8], as well as intratumoral heterogeneity within a patient’s tumor [9–11].

As for long-term BrCa prognosis, current 5-year survival rates of nearly 90 % may indicate that local treatment with surgical removal and chemo-radiation therapy or endocrine therapy is relatively effective. However, there is a population of patients whose tumors are resistant to such therapies and might demonstrate a higher rate of tumor recurrence. Tumor recurrences after multimodal systemic treatment approaches (chemotherapy/endocrine therapy) are likely to be driven in part by intratumoral heterogeneity. Therefore, to improve long-term survival in BrCa, a new translational research model that retains intratumoral heterogeneity is essential. Given this need, patient-derived xenograft (PDX) models have emerged as an useful tool for translational research and preclinical testing in general oncology including BrCa. PDXs are generated by xenografting a small fragment of human cancer tissue, obtained from surgical resection or biopsy, into immune-deficient mice. The key feature of PDX models in cancer research is that each PDX represents the biological characteristics and genetic diversity of the original tumor, which allows it to be used as an ideal model for testing in a diverse pool of human cancer patients. In as early as the 1980’s, using PDX models of small cell lung cancer (SCLC), Fiebig HH et al demonstrated that the response of PDX models to cytotoxic agents was highly correlated with the original patient’s clinical response [12]. Recently, with increased awareness of the importance of tumor heterogeneity to response to treatments and outcome, as well as increased enthusiasm for personalized medicine, there has been an explosion in reports on the use of PDX models in the field of BrCa research. Herein, we review the current state of patient-derived xenograft models in BrCa. We also discuss current and future uses of PDX models in translational research and the preclinical setting.

Generation of PDXs

The techniques to efficiently generate PDX models

The techniques to generate PDX models in mice from primary or metastatic human cancer tissue have been described extensively in the literature [13, 14]. Briefly, diced fragments of the tumor are implanted into immune-compromised mice immediately after surgical removal from the patient. In some cases, cancer cell suspensions from pleural or peritoneal fluid were used for inoculation. The tumor can be passaged by incising and implanting tumor fragments into the next animal.

Some ingenuity has been exercised to improve the rate and speed of engraftment, and to generate models that better mimic human tumors [15–18]. It has been claimed that the engraftment rate is better when solid pieces of tumor tissue are implanted compared to tumor cell suspension following tumor digestion. Addition of irradiated or non-irradiated human fibroblasts with implanting cancer tissue has been reported to improve the engraftment rate [15]. On the other hand, the use of estradiol pellets in the host increased engraftment rates of breast cancers, while co-implantation with human fibroblasts decreased engraftment rate in this setting. It has not been clarified why human fibroblasts would stimulate tumor growth in normal human mammary tissue, but inhibit estradiol-enhanced growth in the PDX [19]. Interestingly, tumor tissue from a metastatic site has a higher engraftment rate in PDX models as compared to their localized primary tumors [20–22]. It is important to note that few studies have directly compared various implantation methods, thus the most appropriate method to generate PDX models with high levels of engraftment take rate (or survival?) remains unclear. In terms of the host animal, severely immunosuppressed mice, such as non-obese diabetic/severe combined immunodeficiency disorder (NOD/SCID) or NOD/SCID/IL2 λ -receptor null (NSG) mice are preferable for establishing PDX models due to higher engraftment rates. However some researchers have reported that implantation in NOD/SCID versus NSG mice have similar take rates [19].

Optimal xenograft site

The anatomical location of grafting of the tumor tissue has been reported to influence the success of engraftment. Utilizing syngeneic models where murine mammary adenocarcinoma cells were implanted into immune intact mice, we have found that genetic profiles of ectopic tumors are considerably different from orthotopic tumors, and orthotopic tumors better model human cancer progression [23–25]. Indeed, orthotopic implantation of BrCa tumor has been described to be more translational, as the tumor develops in the same anatomical microenvironment [26]. Similarly, orthotopic PDX models have been used in most recent publications for BrCa PDX models [27–40]. Other than breast cancer, ectopic implantation of tumor tissue under the renal capsule has been shown to substantially increase engraftment rates in several cancers such as ovarian and lung [18]. Multiple reports demonstrated that tumor implantation under the renal capsule yielded an impressive 90 % engraftment rate in non-small cell lung cancer (NSCLC) as compared to 25% in subcutaneous implantation site [16, 17]. Implantation under the renal capsule also shortened time to engraftment, which is a profound advantage if one is to implement real time PDX

data for personalized cancer treatment for a patient. However, these results were not obtained from a single comparative study.

Advantage of PDX models over conventional xenograft models in translational research

Although conventional xenograft models that implant established cancer cell lines into immune-deficient mice are convenient, relatively low cost, and easy to use, it does have limitations in preclinical drug development. Cell lines that they have been grown on plastic and then been implanted in vitro are unlikely to represent the entire spectrum of heterogenous cancer. The predictive value of conventional xenograft models with regard to clinical outcome is poor, regardless of whether testing is performed using cell lines grown in vitro or in vivo in host animals [41]. One of the underlying causes may be alterations in the biological properties of the cells, such as gain and loss of genetic information, alteration in growth and invasive properties, loss of specific cell populations, and selection of cell population. These can occur during the process of establishing cancer cell lines, resulting in profound changes in the biological behavior of the new line from the original tumor cells. Given the fact that cell lines are grown on plastic culture, it is not difficult to imagine that the biologic conditions of the cancer cell can change from those of the host during the process of adaptation to in vitro growth conditions. These changes include modifications in key properties such as genetic content, epigenetic signature, invasive capabilities, maintenance of a heterogeneous cell population and the reliance on specific growth and survival pathways [42]. A study in SCLC compared the gene expression profiles between a patient's original tumor, its PDX tumor, and cell lines developed from the patient's tumor grown both in vitro and in vivo as a conventional xenograft model [43]. The results demonstrated that while the genetic expression profile of the PDX tumor was similar to the original cancer, cell lines developed from the PDX tumor presented a different profile that was not restored by in vivo subcutaneous propagation in mice. This study also showed loss of a group of tumor-specific genes expressed in both the primary SCLC tumor and PDX tumor during the transition to cell culture, which were not regained when the cells were reestablished as xenografts. Most likely these changes in genetic profiles are not specific to SCLCs, but related to the selection process and adaptation to in vitro culture conditions [43].

The succession of tumor characters in PDX models after mouse-to-mouse passage

Morphology and tumor pathology—Several studies have demonstrated that PDX models retain the principal morphological and functional characteristics of donor tumors, including the fine tissue structure and subtle microscopic details such as gland architecture, mucin production or cyst development. Most studies demonstrated that clinically relevant markers such as estrogen receptor (ER) and HER2 expression of PDX tumor are concordant with the source tissue by histological analysis [35, 31, 44, 19, 33, 40, 27, 28, 39, 20]. For instance, DeRose YS, et al. demonstrated that the expression of clinically relevant molecular markers, such as ER, PR, and HER2 of primary source tumors were conserved in each PDX tumors of all 12 cases in their study [45]. They also found that the majority of the primary-PDX tumor followed similar metastatic patterns of those seen in the original patients [45]. These clinically relevant markers, such as ER, PR and Her2, as well as tumor morphology have critical importance in cancer progression and prognosis, which is evident from the fact that they are now part of TNM Cancer Staging for breast cancer in the (latest) 8th edition of

the AJCC Staging Manual. Therefore, preservation of these markers make PDX models a clear departure from the rest of the animal models in preclinical testing.

Gene expression—Most studies show good concordance of gene expression between the original donor tumor and the PDX tumors derived from them [45, 46, 19, 34, 37, 40, 27, 32, 39, 47]. Analysis of gene expression profiles, using genome sequencing, microarray, and quantitative polymerase chain reaction, demonstrated that there were few substantial changes between donor tumor and their corresponding PDX. Analyses of copy number alterations (CNAs) and exome sequence displayed concordance between paired samples, with a trend towards higher frequency of genomic alterations in the PDX models, likely as a result of increased human tumor DNA extracted from the PDX model. A recent analysis of whole genome sequencing of BrCa patients' original donor tumors and PDX tumors demonstrated that PDX tumors have relatively stable genomes without a significant accumulation of DNA structural rearrangements. However various numbers of PDX-specific somatic alterations were observed at the single-nucleotide level, although they were rarely functionally significant. Variant allele frequencies were often preserved in the PDX models, demonstrating that clonal representation can be transplantable through the PDX passages [33]. Several studies have shown that intrinsic breast cancer phenotypes are well represented in PDX tumors, and they are in concordance with the original tumors using gene expression profiles [45, 46, 19]. For instance, histological and immunohistochemical analyses using hormone receptor markers (ER, PR, HER2), as well as TP53, epidermal growth factor receptor (EGFR, ErbB1), cytokeratin 19 (CK19), cytokeratin 5 (CK5), and Ki67, showed strong concordance between PDX tumors and the original tumor [19]. Interestingly, PDX tumors retained all of the mutations identified in the original tumor, and displayed a mutation enrichment pattern that resembled metastatic disease. For instance, several mutations detected in the breast PDX tumors were also observed in the derived patient's brain metastases. Differential mutation frequencies and structural variation patterns in metastasis and xenograft compared with the original primary tumor indicate that metastatic tumors may arise from a minority population of cells contained within the primary tumor [48]. The results could suggest that these mutations observed in metastatic site and PDX tumors could be the result of adaption to transplantation into the new microenvironment (i.e. metastatic sites), which could also be present in the original tumor below detectable limits.

Replacement by murine stromal compartment—Another important aspect of PDX tumors that should not be overlooked is the replacement of the human cancer stromal compartment, present in the implanted tumor pieces, by murine stroma throughout the growth of tumor in the mice. The current consensus is that the PDX models are stable and will yield consistent results for preclinical studies after third passage in mice. By the third passage the PDX tumor stroma completely includes the murine extracellular matrix, cancer associated fibroblasts, blood vessels and inflammatory and immune infiltrate cells such as leukocytes and macrophages. The PDX murine stroma probably results in changes in paracrine regulation of the tumor as well as in physical properties such as interstitial pressure that may or may not limit the study of agents directed against the tumor stromal compartment [49, 50]. Namely, human stromal cells are gradually replaced by murine equivalents upon engraftment in the mouse, suggesting that implanted human cancer cells

retain the ability to recruit murine accessory cells to the tumor niche [51]. The gene expression profile related to the stromal compartment and immune function of patient tumor was less represented in the PDX models, presumably due to replacement of the human stroma by murine elements [49–51].

Application of PDX models; drug screening and biomarker development in BrCa

Table 1 shows a brief summary of recent applications of PDX models for drug screening and biomarker development, as well as defining metastatic mechanisms in BrCa. Recently, several publications have demonstrated the utility and availability of BrCa PDX models in the preclinical setting [39, 38, 32, 28, 40, 27, 52, 37, 33, 29, 36, 35, 34, 31, 30, 53, 54, 44]. The potential applications of PDX models are vast and include the preclinical evaluation of therapeutic efficacy of new treatment targets, novel combinations of therapy, and optimal treatment schedules. The possible applications also include the molecular analysis of tumor response, the identification of biomarkers indicating drug responder or non-responder, and mechanisms of drug resistance.

Many agents and compounds are advanced to large phase III studies that consume considerable resources, only to end up failing because of a lack of efficacy. Part of the reason for these poor results is that conventional preclinical models utilized to screen new agents for clinical development have poor clinical predictive value. To resolve these clinical issues, PDX models have emerged as a preclinical tool for evaluating drug response. It has been repeatedly shown that PDX models demonstrated similar levels of therapeutic activity compared to the clinical response of patient's tumor to therapeutic intervention. Some studies report remarkable one to one concordance between individual donor patient's breast cancer tumor and its PDX tumor in response to conventional anticancer agents [55, 19]. Thus, PDX models have recently been used to evaluate personalized drug treatments. The PDX model has been used to test multiple treatments for a patient's triple-negative metastatic breast cancer, to choose the most effective drug combinations to provide better outcomes [56]. Studies have compared the response of drug treatments of PDX models from different passages and shown stable response rates across multiple passages, further supporting the phenotypic stability of these models [57, 58].

Another interesting study using PDX models by Lawson D et al. demonstrated that progression to high metastatic burden was associated with increased proliferation and MYC expression, which could be attenuated by treatment with cyclin-dependent kinase (CDK) inhibitors. They proposed a hierarchical model for metastasis, in which metastases are initiated by stem-like cells that proliferate and differentiate to produce advanced metastatic disease using PDX models [32].

Limitation of PDX model

While the incorporation of PDX models in cancer research brings some exciting improvements as detailed above, PDXs have important limitations that need to be addressed to improve availability of PDX models for translational cancer research and pre-clinical investigations. Technical issues that need to be clarified for wide use of PDX models are: (1) optimal implantation site, (2) time course to generate tumors, (3) higher engraftment rate,

and (4) replacement of human stroma with murine stroma. (1) It is important to define the best strategy of engraftment in mice (subcutaneous, subrenal or orthotopic implantation) for BrCa. (2) Delay between engraftment time in mice and optimal treatment schedules for patients is also a limiting factor for use of PDX models for real-time personalized medicine applications. The delay especially pertains to the time course to generate stable PDX models. It normally takes 4–8 months (several tumor passages) to develop a PDX model ready for a preclinical study, which is much longer than patients can normally afford. (3) As described above, another limitation is the engraftment failure rate, which is still high for some tumor types with particular phenotypes [59, 47, 60]. This is a well-known problem for hormone receptor positive BrCa, which has a low engraftment success rate. For application for personalized or precision medicine, it may be necessary to improve tumor take rates to an acceptable level (60–70%). (4) Aside from clonal dynamics driven by intrinsic differences in genetic or epigenetic background, intratumoral heterogeneity can be influenced by tumor-extrinsic factors in the autonomous compartment including the microenvironment from host cells of mice. Cellular interactions with the extracellular matrix can profoundly alter not only gene expression but also tumor cell behavior [61, 62]. Tight regulation of the extracellular matrix is lost during cancer development and tissue architecture degrades. A recent study demonstrated that extracellular matrix-dependent signaling confers dynamic switching between TGFBR3 (transforming growth factor b receptor 3) and JUND (jun D proto-oncogene) related expression signatures. Extracellular matrix driven oscillations between signaling pathways such as those described could have profound effects on cancer progression [62]. In PDX models, Matrigel is often used to increase the engraftment rate. However, it should be recognized that this is a murine basement membrane extract and suitable synthetic human alternatives are now available [63, 64]. Therefore, it should also be recognized that Matrigel from murine sources can be a source of infectious murine viruses. Moreover, the presence of growth factors in Matrigel may favor the engraftment of one cell type over another.

Future perspective

Based on the data described above, PDX models may play pivotal roles in drug-response studies. To identify populations of patients that may be more responsive to new agents, PDX models can be used to discover new biomarkers, as PDXs recapitulate tumor diversity in the human disease including phenotype and genome expression of the individual patients. PDX models representing a population of human tumors may be used for preclinical studies of novel therapeutic agents in parallel to phase I safety and pharmacological trials [65]. Moreover, PDX models should also be used in “basket trials”, because PDX models could be used to define groups of tumor types of interest, based on molecular and genetic traits. One of the advantages of the existing PDX models is that they have been extensively characterized at the histological, molecular and genomic levels. Recently, a few reports have demonstrated feasibility of PDX models for exploring circulating tumor DNA (ctDNA) or circulating tumor cells (CTCs) in solid cancers [66, 67]. We believe that PDX models could be utilized in preclinical research of personalized liquid biopsy targeting for circulating nucleic acids such as ctDNA or microRNA. Utilizing conventional xenograft model with cancer cell inoculation, circulating microRNA was shown to be feasible for detection of solid tumor in 2008 [68]. As described above, PDX models should reflect tumor

heterogeneity and its dynamics better than conventional xenograft models, thus it should be more appropriate tool to explore circulating nucleic acids, such as ctDNA or microRNA, which can be utilized as cancer biomarkers in preclinical research.

On the other hand, recent studies have demonstrated the utility of antitumor immunotherapy or intratumor immune response as a prognostic biomarker in BrCa [69–72]. Namely, tumor cells are broadly thought to be antigenic with point mutations in coding exons in a tumor resulting in various representations of neoantigens. Targeting these neoantigens can lead to lymphocyte invasion into the tumor and significant CD8+ cytotoxic T-cell infiltration and tumor cell death. However, most tumors eventually progress and evade the immune system - often through inhibitory effects to antitumor immunity by immune checkpoints proteins such as CTLA-4 or PD-1 on the surface of T cells. While cancer immunotherapy has emerged as a new therapeutic choice in several cancers in the clinical setting, only a few reports have focused on investigation of cancer-immune system interaction in BrCa [71, 69, 72]. Thus, so far, cancer immune therapy related to checkpoint inhibitors in BrCa has not been clarified. One key limitation of PDX models is the need to use immunodeficient host strains for tumor engraftment and propagation. Therefore, PDX models currently have limited utilization for investigation on cancer immunotherapies such as immune checkpoint blockade therapy.

Recently humanized NSG mice (hu-NSG), which is defined as a mouse being engrafted with human primary hematopoietic cells that generate a functional human immune system, is a humanized mouse model that allows the generation of peripheral human immune responses, particularly CD8+ Cytotoxic T lymphocyte (CTL) responses, and serves as an effective model for studying drug and gene-based therapies. Generally, the most robust reconstitution of the human immune system in immunodeficient mice is seen when bone marrow or cord blood-derived human hematopoietic stem cells or progenitor cells (generally CD34+ stem cells) are co-engrafted followed by patient-derived tissue or disease models induced to the mice. CD34+ cells could potentially be harvested from patient's bone marrow or cord blood to reconstitute a functional and patient-matched immune system in mouse models. An immune system could mature in the mice and human immune cells would undergo central tolerance to mouse antigens during development [73–76]. Several reports demonstrated that establishment and utility of humanized mice by allogeneic immune cells in the context of a mouse with a human immune system can be used to explore the response of the immune system to infections or answer other tumor-immunological questions [75, 73]. However, it will be a challenging field to establish PDX models in humanized mice bearing human immune systems as mentioned above.

Conclusions

The field of oncology is rapidly evolving from 'all comers' approach to cancer therapy to an era in which patients' tumors are molecularly profiled in greater detail to select the most appropriate treatment, so-called "precision medicine." It is well known that colorectal cancer, non-small cell lung cancer, and BrCa tumors are now routinely genomically profiled to determine treatment strategy in clinical practice. While these approaches represent a significant advance in translational cancer research, further advances are required. PDX

models, and in the near future, possibly humanized PDX models, could address tumor heterogeneity and diversity for better treatments and outcomes.

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Abbreviations

BrCa	Breast cancer
PDX	patient-derived xenograft
SCLC	small cell lung cancer
NSCLC	non-small cell lung cancer
NOD/SCID	non-obese diabetic/severe combined immunodeficiency disorder
NSG	NOD/SCID/IL2 λ -receptor null
CAN	copy number alterations
CTL	Cytotoxic T lymphocyte

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Table 1

Authors	Year	Tissue source ¹	Subtype ² (n)	Supportive conditions ³	Implantati on site ⁴	Take rate (%)	Concordance method	Findings	Ref.
Ibrahim YH	2012	P, M	T(3)	E2	SQ	NR	NR	Preclinical benefit of PARP inhibitors on TNBC.	30
Oakes SR	2012	P	T(3), L(1), H(1)	E2	MFP	28/112 (25)	Histological	BH3 mimetics can be predictor of chemosensitivity for primary BrCa.	35
Ma CX	2012	P, M	T(3)	Fib	MFP	NR	PAM50, WGS	Therapeutical benefit of targeting Chk1 in p53-deficient TNBC.	34
Kabos P	2012	P, E	T(2), L(8)	E2, Mat	MFP	10/24 (42)	Histological	PDX retain hormone receptor heterogeneity and unique estrogen-dependent gene signatures.	31
Romanelli A	2012	P	T(9), H(1),	E2	SQ	NR	NR	Aurora kinases could represent a new effective approach for TNBC.	36
Vaillant F	2013	P	T(17), L(13), P(2), H(5)	E2	MFP	37/ 158 (23)	Histological	Utility of BH3 mimetics for BrCa with BCL-2 as a companion biomarker.	44
Moestue SA	2013	P	T(23), L(17)	NR	MFP	NR	NR	Metabolic biomarkers for response to PI3K inhibition in basal-like BrCa.	53
Qiu M	2013	P	T	NR	MFP	NR	NR	Anti-Notch1 antibody improve the efficacy of docetaxel by directly targeting the cancer stem cell niche.	54
Green JL	2013	P	T (6)	NR	MFP	NR	NR	Paracrine Wnt signalling both promotes and inhibits tumour growth.	29
Schott AF	2013	P, E	T	NR	MFP	NR	Array, RPPA, sequenom	Utility of gamma secretase Inhibitors with docetaxel on BrCa.	37
Zhang X	2013	P, E	T (12), H (3)	E2, Fib	MFP	23/169 (13)	Histological	PDX models serves a renewable, quality-controlled tissue resource for preclinical studies investigating treatment response and metastasis.	19
du Manoir S	2014	P	T (14), L (5), H (3)	E2	SQ	20/129 (15)	Histological, array, PAM50	Tumors producing stable PDXs showed increased expression of several gene signatures such as GGI, wound healing, proliferation, IL8 and VEGF.	47
Zhang H	2014	P, M	T (7)	Mat	MFP	NR	Histological, array sequence	mTOR inhibitors can be effective in TNBC.	40
Cottu P	2014	P, M	L (2)	E2	SQ	NR	Histological, PCR, array	Resistance to endocrine therapies is associated with specific deregulations of ER-mediated gene transcription.	27
Eirew P	2015	P, E	T (6), L (5), H (4)	E2	SQ, MFP, SRC	NR	Histological, WGS	Measurement of genomically defined clonal population dynamics is highly informative for functional studies.	28

Authors	Year	Tissue source ¹	Subtype ² (n)	Supportive conditions ³	Implantati on site ⁴	Take rate (%)	Concordance method	Findings	Ref.
Lawson DA	2015	P, E	T (3)	Mat	MFP	NR	FACS	Initial metastasis are generated by stem-like cells that proliferate and differentiate to produce advanced metastatic disease in BrCa.	32
Simoes BM	2015	P, E	L (23), H (3)	E2, Mat	MFP	NR	NR	Anti-estrogen resistance in is driven by JAG1-NOTCH4-dependent cancer stem cell activity.	38
Zacarias-Fluck MF	2015	P	H (6)	E2	MFP	NR	Histological, array	Senescent cells can contribute to the growth of tumors by providing cytokines in HER2-positive BrCa.	39

¹ P, primary; E, pleural effusions; M, metastatic tissue.
² T, triple negative breast cancer; L, luminal type; H, her2 positive.
³ E2, estradiol; Fib, fibroblasts; Mt, matrigel.
⁴ SQ, subcutaneous; MFP mammary fat pad; SRC, subrenal capsule.