‘Omics Approaches to Explore the Breast Cancer Landscape

Joseph Parsons1,2 and Chiara Francavilla*

1 Division of Molecular and Cellular Function, School of Biological Sciences, Faculty of Biology Medicine and Health, The University of Manchester, Manchester, United Kingdom,
2 Division of Cancer Sciences, School of Medical Sciences, Faculty of Biology Medicine and Health, The University of Manchester, Manchester, United Kingdom

Breast cancer incidence is increasing worldwide with more than 600,000 deaths reported in 2018 alone. In current practice treatment options for breast cancer patients consists of surgery, chemotherapy, radiotherapy or targeting of classical markers of breast cancer subtype: estrogen receptor (ER) and HER2. However, these treatments fail to prevent recurrence and metastasis. Improved understanding of breast cancer and metastasis biology will help uncover novel biomarkers and therapeutic opportunities to improve patient stratification and treatment. We will first provide an overview of current methods and models used to study breast cancer biology, focusing on 2D and 3D cell culture, including organoids, and on in vivo models such as the MMTV mouse model and patient-derived xenografts (PDX). Next, genomic, transcriptomic, and proteomic approaches and their integration will be considered in the context of breast cancer susceptibility, breast cancer drivers, and therapeutic response and resistance to treatment. Finally, we will discuss how ‘Omics datasets in combination with traditional breast cancer models are useful for generating insights into breast cancer biology, for suggesting individual treatments in precision oncology, and for creating data repositories to undergo further meta-analysis. System biology has the potential to catalyze the next great leap forward in treatment options for breast cancer patients.

Keywords: breast cancer, system biology, proteomics, transcriptomics, genomics, organoids, PDX

BREAST CANCER – WHERE ARE WE?

Breast cancer is the leading cause of cancer-related deaths in women worldwide (Bray et al., 2018). It is a heterogeneous disease (Nik-Zainal et al., 2016), commonly separated into Luminal A (LumA), Luminal B (LumB), epidermal growth factor receptor ERBB2/HER2-overexpressing (HER2+), basal epithelial-like (BL) based on gene expression profiles (Sørlie et al., 2001). Breast cancer is currently treated with surgery, radiotherapy, cytotoxic chemotherapy and/or targeted therapies to eradicate viable cancer cells (Fisher et al., 2002).

LumA and LumB breast cancers are both estrogen receptor (ER)-positive (Sørlie et al., 2001). Deregulated ER signaling is associated with cancer hallmarks (Hanahan and Weinberg, 2011). For instance, ER target genes like cyclin-dependent kinase (CDK) 1 or the kinase Src promote...
cell proliferation, invasion and epithelial–mesenchymal transition (EMT) (Stender et al., 2007; Saha Roy and Vadlamudi, 2012). LumB cancers have high expression of the proliferation marker Ki67, which correlates with increased risk of developing distant metastases (Colzani et al., 2014), and reduced expression of the progesterone receptor (PR) (Cho, 2016), which shifts gene expression toward more tumorigenic genes (Mohammed et al., 2015). LumA and LumB tumors are treated using ER antagonists (e.g., tamoxifen), aromatase inhibitors and selective estrogen receptor degraders (e.g., fulvestrant). However, therapeutic resistance may arise through loss of ER expression, mutations in ER or overexpression of alternative breast cancer-driving pathways such as ERBB1/EGFR (Garcia-Becerra et al., 2012; Clarke et al., 2015; Ma et al., 2015). To overcome resistance to traditional ER antagonists targeted therapies against phosphoinositide 3-kinases (PI3K), mammalian target of rapamycin (mTOR), and CDK4/6 have recently been proven beneficial in the clinical setting (Beaver and Park, 2012; Kornblum et al., 2018; Pernas et al., 2018).

HER2 + breast cancers overexpress ERBB2/HER2 (Iqbal and Iqbal, 2014) which promotes proliferation by regulating CDKs and Cyclins (Timms et al., 2002). Additionally, HER2 dimerization with EGFR induces activation of mitogen-activated protein kinase (MAPK), c-Jun N-terminal kinases (JNK), and phosphoinositide phospholipase C (PLCγ) signaling pathways resulting in increased cell proliferation, migration and apoptosis resistance (Masuda et al., 2012). HER2 + breast cancers are treated with targeted agents such as trastuzumab, pertuzumab, and neratinib. Trastuzumab is an antibody which inhibits HER2 dimerization, promotes natural killer cell recruitment to tumors and stimulates ubiquitin-dependent HER2 degradation (Vu and Claret, 2012; McCann and Hurvitz, 2018; Schmid et al., 2018; Vikas et al., 2018). Therapeutic resistance to trastuzumab occurs via HER2 dimerization with other ERBB family members or constitutive HER2 activation (Vu and Claret, 2012).

BL breast cancers do not generally express ER, PR or HER2 (Milioli et al., 2017), like triple negative breast cancers (TNBCs) (Lehmann et al., 2016). BLs are highly heterogeneous and include basal-like 1-2, claudin-low, and immunomodulatory subgroups (Garrido-Castro et al., 2019). BLs have a highly proliferative and invasive phenotype with high risk of relapse in early breast cancer (Fallahpour et al., 2017). BLs are typically treated by chemotherapy and radiotherapy (Wahba and El-Hadaad, 2015) although recent advances have led to novel treatment opportunities for BL cancer patients. For instance, immunomodulatory BLs can be treated with immune checkpoint programmed cell death protein 1 (PD-1) and poly (ADP-ribose) polymerase (PARP) inhibitors (McCann and Hurvitz, 2018; Schmid et al., 2018; Vikas et al., 2018).

Two major challenges in breast cancer treatment are therapeutic resistance and the formation of metastasis to secondary sites (lung, bone, lymph nodes, brain, and liver) inevitably leading to patient mortality (Minn et al., 2005). As 10 year survival for metastatic breast cancer patients remains below 5% (Kontani et al., 2014) and response to targeted therapies varies from 15 to 40% for all subtypes (Bartsch et al., 2007; Haque and Desai, 2019) the need for novel therapeutic options for breast cancer patients remains a priority.

Here, we will describe several models that have contributed to knowledge of breast cancer biology and the repertoire of currently available therapeutic targets. Thereafter, we will introduce system biology-based approaches and finally discuss how their integration with traditional models is revolutionizing breast cancer translational research.

MODELS TO STUDY BREAST CANCER

Cell Lines
Breast cancer has been traditionally studied using immortalized cell lines derived from patient samples (Holliday and Speirs, 2011) which are easy and inexpensive to grow. These cell lines express biomarkers of the different molecular subtypes of breast cancer (Dai et al., 2017) and recapitulate some parent tumor characteristics including drug responses (Holliday and Speirs, 2011) and transcriptomic profiles (Neve et al., 2006). Cell lines have enabled major discoveries in breast cancer research, such as the identification of oncogenes (Elenbaas et al., 2001) and drivers of metastatic tropism (Minn et al., 2005). However, breast cancer cell lines have increased gene copy number variations compared to primary tumors (Larramendy et al., 2000), lack the in vivo microenvironment (Vincent et al., 2015), and do not maintain primary tumor heterogeneity (Dai et al., 2017; Liu et al., 2019) (Figure 1A).

Organoids
Organoids are three dimensional (3D) cell cultures which mimic healthy tissues and cancer lesions (Xu et al., 2018). Organoids are usually grown in matrices such as Matrigel™, collagen or peptide hydrogels which aim to recapitulate the breast microenvironment (Djomehri et al., 2019). The group of Mina Bissel in the ’80s began to investigate how organoids were a better model for studying breast tissue compared to 2D cell culture (Weaver et al., 1995). More recently, primary and metastatic organoids have been developed which accurately recapitulate parent tumor characteristics including histopathology, genomic abnormalities and drug responses (Sachs et al., 2018). Organoids are easy to modify, can be propagated for up to 3 months (Fatehullah et al., 2016), and allow drug screening (Dutta et al., 2017). Recently, the issue of availability of primary patient samples for laboratories without access to biobanks has been solved by the creation of living biobanks of frozen organoids (Dutta et al., 2017). Organoids can be used as models to study different breast cancer subtypes and to identify potential novel therapeutic targets. Organoid are better models than 2D cultures to analyze drug response due to a more representative microenvironment and selection for stem-like cells, like those responsible for metastatic initiation (Velasco-Velazquez et al., 2011; Imamura et al., 2015). Despite these promising characteristics for breast cancer translational research, organoids lack components of the in vivo microenvironment and may suffer for counterselection of hyperproliferative cells (Fujii et al., 2016; Weeber et al., 2017) (Figure 1B).
| Model          | Advantages                                                                 | Disadvantages                                                                 | Major discoveries                                                                 |
|---------------|-----------------------------------------------------------------------------|-------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| A  CELL LINES | - Cheap and easy to culture                                                 | - Increased mutation rate compared to tumours                                 | - Identification of the role of oncogenes                                         |
|               |                                                                             | - Clonal population does not represent tumour heterogeneity                    | - Identification of drivers of metastatic tropism to bone, brain and lung         |
|               |                                                                             | - Heterogeneity within cell lines used in different labs                       | - Identification of genetic aberrations involved in resistance to targeted therapies |
| B  ORGANOIDS  | - Similar structures to those seen in the breast (such as milk-producing acini) | - Availability of the initial patient samples and variability in the culturing systems among laboratories | - Creation of living biobank in which tumour tissue can be expanded whilst maintaining genomic and transcriptomic profiles of the original sample |
|               | - Similar histopathology, genomic abnormalities and drug responses of tumours | - Lack of stromal and immune components                                       | - Recapitulation of drug responses                                                |
|               | - Suitable for medium throughput drug screens                               | - Counterselection for hyperproliferative cells                               | - Modelling of breast cancer tissue characteristics                              |
| C  GEMMs      | - Presence of natural micro-environment and immune system                    | - Extensive breeding time and costs                                           | - Identification of mutations                                                     |
|               | - Partial recapitulation of human breast cancer subtypes                     | - Non-physiological levels of oncogenes                                       | - Identification of cell of origin                                                |
|               |                                                                             | - Genetically different from human tumours and rarely mimicking clinical metastases | - Identification of novel drugs combination or mechanisms of resistance           |
| D  PDX        | - Maintenance of the genomic, transcriptomic and proteomic profiles of tumours through multiple passages | - Use of immunocompromised mice to prevent rejection of human cells           | - Creation of a bank of tumour tissue which can be passaged and maintained in different laboratories |
|               | - Maintenance of metastatic tropisms and heterogeneity of patient tumours    | - The mouse microenvironment may result in the clonal selection of the more aggressive cells of the patient sample | - Identification of the contribution of heterogeneity to breast cancer progression |
|               | - Formation of spontaneous metastases                                       |                                                                              | - Identification of drug combinations to overcome resistance                     |
| E  ‘OMICs APPROACHES | - Unbiased analysis of the DNA, RNA and protein landscape starting from any sample | - High costs in terms of sample handling and starting amount, instrumentation and time for data analysis and integration | - Identification of potential novel biomarkers, drivers, and therapeutic targets  |
|               | - Rapid and robust data generation                                          | - Poor correlation between ‘omics approaches (e.g. transcriptomics vs. proteomics) | - Identification of specific mutations linked to drug responses                    |
|               | - Creation of data repositories that can be used for other studies or for validation by other researchers | - Single cell analysis held great potential, but is still under development     | - Identification of basal-like subsets                                             |

**FIGURE 1** Models and methods to study breast cancer. Summary of the advantages (left column) and disadvantages (middle column) of existing breast cancer models (A–D) and ‘omics technologies (E) to study breast cancer. Right column reports a brief summary of how different methods and models have contributed to major discoveries in the field of breast cancer.
Genetically Engineered Mouse Models (GEMMs) and Syngeneic Mouse Models (SMMs)

In vivo modeling of breast cancers generally entails inducing oncogene expression (e.g., Erbb2) or knocking out a tumor suppressor gene (e.g., p53) in mice. Examples include the mouse mammary tumor virus (MMTV) promoter-driven or the 4T1-based SMMs (Holen et al., 2017). GEMMs include a natural (mouse) microenvironment and immune system, and partially mimic all human subtypes save luminal cancers (Pfefferle et al., 2013; Holen et al., 2017). However, GEMMs involve extensive costs and breeding time, often express supra-physiological levels of the transgene, and can be genetically different compared to their human counterpart (Pfefferle et al., 2013). Only 16 of the 30 most commonly mutated genes in human breast cancers were found to be mutated in a panel of metastatic GEMMs and SMMs (Yang et al., 2017). Although SMMs have higher mutational burden in metastases than in primaries like human breast cancers (Yang et al., 2017; Yates et al., 2017), GEMMs and SMMs rarely mimic clinical metastasis (Holen et al., 2017). In spite of these pitfalls, GEMMs have been instrumental in generating insights into breast cancer biology – e.g., determining that BRCA1 mutant tumors derive from luminal progenitor rather than basal cells (Molyneux et al., 2010) and in testing novel drugs combinations (Jaspers et al., 2013) (Figure 1C).

Patient-Derived Xenografts (PDXs)

Patient-derived xenografts (PDXs), which involve injection of human cancer cells either orthopically in the mouse mammary fat pad or subcutaneously into immunocompromised mice, provide an in vivo alternative to GEMMs (Hidalgo et al., 2014; Holen et al., 2017). They have helped address clinically relevant questions including the contribution of heterogeneity to, and the mechanism of, drug resistance (Byrne et al., 2017). PDXs can be passaged in different mice allowing expansion of patient tissue whilst still maintaining ‘omics profiles of the patient tumor; and they spontaneously metastasize (DeRose et al., 2011; Dobrolecki et al., 2016). Drawbacks for the use of PDXs include the selection of more aggressive cells within the patient sample and the use of immunocompromised mice to prevent tumor rejection. Developing mice with humanized immune systems can help to address this problem (Hasgur et al., 2016), as recently shown for a metastasis model (Rosato et al., 2018) (Figure 1D).

In conclusion, choosing the correct model to study breast cancer depends on several factors including the biomedical question, sample availability, costs, etc. (Figure 1). We envision that future interdisciplinary research will be based on a combination of different models to identify and validate new therapeutic targets for breast cancer treatment with the advent of next generation sequencing and more robust instrumentation, ‘omics approaches, like genomics and proteomics, are becoming more accessible and are increasing the information that can be obtained from breast cancer models. Thus, ‘omics approaches applied to the combination of different models will provide molecular information on a global scale and will identify novel targets.

SYSTEM BIOLOGY APPROACHES TO STUDY BREAST CANCER

System biology based on ‘omics approaches and network science are becoming popular in cancer research (Manem et al., 2018), despite high costs in terms of sample handling, instrumentation, and time for data analysis. Integrating ‘omics approaches allows the unbiased analysis of the whole genome, transcriptome, proteome, or metabolome starting from different types of samples (Figure 1E and Table 1).

Genomics

Next generation sequencing (NGS) allows rapid and relatively inexpensive DNA sequencing covering the whole genome (Park and Kim, 2016). Genomic approaches helped redefine breast cancer subtypes (Cancer Genome Atlas Network, 2012), identify mutational landscapes (Stephens et al., 2012) or single nucleotide polymorphisms (SNPs) as a biomarker of breast cancer susceptibility (Michailidou et al., 2017) or therapeutic response (Kus et al., 2016). NGS has also facilitated the discovery of breast cancer driver mutations (Nik-Zainal et al., 2016), tumor heterogeneity (Yates et al., 2015) and novel therapeutic targets in metastatic disease (Bertucci et al., 2019). Finally, single-cell analysis allowed the study of breast cancer stem cells (Lawson et al., 2015). However, accurate genomic analysis requires large numbers of sequence reads which increases both time and cost.

These discoveries demonstrate the potential for genomics to transform breast cancer treatment (Hamdan et al., 2019). For instance, genomics helped identify patients for clinical trials (Curtis et al., 2012) or high risk individuals through mutation screening in breast cancer susceptibility (BRCA) 1–2 genes (Evans et al., 2008) and contributed to therapeutic decision making (Tsoutsou et al., 2017; Bergom et al., 2019). As an invaluable resource for researchers, the Catalogue of Somatic Mutations in Cancer (COSMIC) has compiled genomic data from breast cancer patient samples and correlated them to cellular functions and drug resistance (Forbes et al., 2017). Finally, genomic analysis for the early identification of tailored therapy for cancer patients has been made possible with the development of the Cancer Genome Atlas (TCGA)1. We envision that TCGA and COSMIC databases will revolutionize cancer patient diagnosis and treatment (Ashton-Prolla et al., 2015). This is already being realized in the MOSCATO trial where druggable genomic aberrations were identified and targeted in patients (Massard et al., 2017).

In addition, cell-free/circulating tumor DNA (cf/ctDNA) can be useful in monitoring clonal evolution and residual tumor presence following treatment (Buono et al., 2019). However, as ctDNA usually comprises 180–200 bp fragments from apoptotic cells, there are varying degrees of success in identifying useful biomarkers with high sensitivity (Sefrioui et al., 2015). Despite this, serial screening for mutations in ctDNA has allowed metastatic detection 8 months before clinical presentation (Garcia-Murillas et al., 2015).

1 https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga
Together with genomics, epigenomics (the study of DNA modifications and their impact) is also providing novel markers for breast cancer prognosis (Davalos et al., 2017) and for detection of metastasis (Legendre et al., 2015). Epigenomics has begun to illuminate the link between menopause and lifestyle factors with breast cancer risk and so may provide prognostic utility in future (Crujeiras et al., 2017).

**Transcriptomics**

Transcriptomics uses microarrays, which quantify a set of predetermined sequences, and RNA sequencing (RNA-Seq), which uses high-throughput sequencing to capture all sequences to determine the quantity of a transcript (Lowe et al., 2017). These approaches have been used to classify breast cancer molecular subtypes in cell lines (Neve et al., 2006) and patient-derived samples (Wu et al., 2018; Jiang et al., 2019), to compare primary breast cancers and their metastases (Vareljila et al., 2018), and to visualize phenotypic features of breast cancer cells in 3D culture (Chung et al., 2017; Azizi et al., 2018), potentially providing a mechanism to inform immunotherapeutic decisions.

As transcriptomics does not provide information on the expression, post-translational modifications (PTMs), or activation status of proteins it is less informative than proteomics for novel therapeutic target discovery. Recent advancements in

**TABLE 1 |** A selection of single- and multi-‘omics-based breast cancer studies that have contributed to major discoveries in the field of breast cancer research where method strengths and weaknesses are reported.

| Study | Topic area | ‘Oomics approaches | Method strengths | Method weaknesses | Major discoveries |
|-------|------------|--------------------|-----------------|------------------|------------------|
| Nik-Zainal et al., 2016 | Novel Breast Cancer Drivers | × | The whole genome sequence can be determined relatively cheaply in less than a week | Sequences must be read many times to account for inaccuracies in sequencing analyzers | Five novel cancer genes were identified. A total of 93 genes were suggested to contain breast cancer driver mutations |
| Playdon et al., 2017 | Breast Cancer Risk | × | This technique is dependent on serum samples which are far easier to obtain than biopsies needed for other ‘omics techniques | Controlling patient diet is very difficult | Three metabolites were found to be associated with increased breast cancer risk |
| Varešlija et al., 2018 | Novel Therapeutic Targets | × × | Combining DNA and RNA sequencing allows mutations to be connected to chromatin remodeling and gene expression | RNA integrity is compromised by the process of formalin fixing due to cross-link formation | RET and HER2 were found to be potential therapeutic targets for breast cancer brain metastases |
| Huang K. L. et al., 2017 | Novel Therapeutic Targets | × × × | Proteomic isobaric labeling methods allow multiple samples to undergo relative quantification reducing variability | Large amounts of starting protein is required for phospho-proteomics. Also proteomic labeling reagents are very expensive | Novel therapeutic targets previously undiscovered at the genomic, transcriptomic or proteome level were identified at the level of the phosphoproteome in PDX models |
| Massard et al., 2017 | Informing Clinical Therapeutic Decisions | × × | When tumor cell population is low in a biopsy, targeted sequencing of known cancer genes can still be used to search for actionable targets without having to purify the epithelial population | Extensive analysis is required to determine if a mutation is actionable. Also biopsies are often sent to pathologists before freezing so the molecular profile may be changed by the time the tissue is frozen | The treatment of 199 patients was based on an actionable genomic alteration which was found using DNA and RNA sequencing In 33% of patients, progression-free survival was significantly increased and in 11% there was objective response |
| Mertins et al., 2016 | Breast Cancer Signaling | × × | In situations where mutations produce unpredictable consequences, e.g., altering splice variants, proteogenomics can identify single amino acid variants and link these to mutations | Proteins which are missing in one or more replicates of a proteomic experiment are often excluded despite the fact the protein may have been present below the detection threshold | A number of highly phosphorylated kinases were identified that were not seen as potential therapeutic targets at the genomic level. Also the impact of mutations was traced to the signaling level to identify therapeutic targets, e.g., CEN34 loss was associated with EGFR upregulation, highlighting how this loss could be druggable |
| Johansson et al., 2019 | Breast Cancer Subtypes | × × × × | Integrating ‘omics technologies allowed the mRNA-based subtypes to be expanded to a more clinically useful resource | Tumors are heterogenous and so ‘omics data from one part of a biopsy may not be representative of the whole tumor | Breast cancer subtypes (Sorlie et al., 2001) were validated at a multi-omic level. Basal-like tumors were separated into two clusters that could inform therapeutic decisions |

G, genomics; T, transcriptomics; P, proteomics; M, metabolomics.
single cell analysis may open a new era in breast cancer research to identify drivers, biomarkers, and novel therapeutic targets (Hong et al., 2019).

In the clinic, analysis of mRNA expression of gene subsets, involved in ER signaling, HER2 signaling, proliferation and invasion, is already used to predict relapse and determine whether patients would benefit from neoadjuvant chemotherapy (Vieira and Schmitt, 2018). Furthermore, as patients with elevated expression of a migratory mRNA signature had worse overall survival than those with a proliferative mRNA signature and so responded significantly better to chemotherapeutics that targeted the cytoskeleton (Nair et al., 2019) transcriptomics has the potential to inform chemotherapeutic decisions in future.

As patient tumor biopsies are typically formalin fixed and paraffin-embedded (FFPE), a preservation procedure that reduces RNA integrity (von Ahlfen et al., 2007), fresh frozen tissue collection should become the standard procedure for mRNA expression to inform clinical decisions.

**Proteomics**

Proteomics studies the expressed proteome and its PTMs by mass spectrometry (MS), protein microarrays, and, more recently, mass cytometry. Advances in samples handling, instrumentation, and data analysis now provide unprecedented insights into the abundance and function of the (modified) proteome (Doll et al., 2019). Proteomics can assess tissue or blood samples, thus lending itself to clinical applications (Mardamshina and Geiger, 2017). For instance, specific serum biomarkers have been discovered by proteomic studies (Li et al., 2002; Raso et al., 2012), potentially providing an early diagnosis signature (Saadatmand et al., 2015). Correlation between RNA or gene copy number with protein expression is rather low (Mertins et al., 2016; Johansson et al., 2019) thus analyzing the patient proteome holds promise for identifying novel preventative or therapeutic targets not previously identified at the genomic or transcriptomic level. This idea is supported by the fact that currently used anti-breast cancer drugs predominantly act against proteins.

MS-based proteomics has been used to characterize cell lines (Huang F. K. et al., 2017), to reveal novel layers of breast cancer classification (Tyanova et al., 2016; Yanovich et al., 2018), and to identify proteins involved in drug resistance (Liu et al., 2006). Furthermore, phosphoproteomics that identify phosphorylated proteins (von Stechow et al., 2015) has been used to connect somatic mutations to signaling (proteogenomics) (Mertins et al., 2016), to identify kinases signatures in TNBC (Zagorac et al., 2018), and to map drug targets for personalized treatments (Pierobon et al., 2018). These discoveries have diagnostic and prognostic potential which is worth further exploring and implementing in the clinic when phosphoproteomics methods will become common practice.

An alternative to MS-based proteomics is provided by mass cytometry where single cells are probed with metal ion-labeled antibodies and then samples are analyzed by time-of-flight mass spectrometry (Leealatan et al., 2017). In breast cancer research this technology has been recently used to identify cell types and immune infiltrates within a tumor (Wagner et al., 2019). However, this method remains limited by antibody availability. Similarly to transcriptomics, phosphoproteomics is also limited by the availability of fresh frozen tissue as the phosphoproteome is substantially altered by FFPE preservation (Wakabayashi et al., 2014).

In conclusion, analyzing the proteome and phosphoproteome of patients at different breast cancer stages will help identify signatures for personalized treatments, ideally starting from liquid biopsies. In future proteomics may be used to follow the response to treatment by analyzing changes in patient proteome so to adapt the therapeutic plan.

**Metabolomics**

Metabolomics is the system-wide identification of endogenous metabolites from bodily fluids in a targeted or unbiased manner (Silva et al., 2019). Metabolomics has been used to correlate changes in metabolism with proliferation rate in breast cancer cells (Jerby et al., 2012), to cluster tumor subtypes (Haukaas et al., 2016), to analyze the lipids content in breast cancer cells (Lisa et al., 2017), and to correlate nutrients with breast cancer risk (Playdon et al., 2017). More recently, this approach has begun paving the way for the identification of metabolic-state specific biomarkers for breast cancer diagnosis (Jasbi et al., 2019). Therefore, metabolomics will allow further insights into correlation between metabolism, epigenomic and proteomic alterations and breast cancer progression or treatment.

**Data Integration**

The contribution of each aforementioned ‘omics technology to the understanding of breast cancer biology and to the discovery of novel targets or biomarkers has been substantial. Integrating these approaches is predicted to be even more powerful (Chakraborty et al., 2018; Manem et al., 2018) (Table 1). For instance, a genomic/transcriptomic/proteomic combined approach has confirmed the existence of the known molecular subtypes (LumA, LumB, HER2+, and BL) of breast cancer (Cancer Genome Atlas Network, 2012) as well as allowing identification of novel therapeutic targets in PDX models (Huang K. L. et al., 2017). Recently, a comprehensive analysis of clinical, genomics, and transcriptomics data has uncovered the TNBC landscape (Jiang et al., 2019). Proteogenomics has challenged the way in which somatic mutations contribute to signaling changes (Mertins et al., 2016), highlighting the need of both these analyses to confirm the therapeutic importance of a genetic alteration. For instance, patients lacking HER2 amplification were found to have enriched HER2 signaling (Pierobon et al., 2018), underlining the importance of analyzing changes in signaling to plan the correct therapeutic approach. With the development of single cell analysis in genomics, transcriptomics and proteomics (Linnarsson and Teichmann, 2016; Hong et al., 2019; Marx, 2019; Wagner et al., 2019) there are opportunities to better understand breast cancer heterogeneity and the role of the microenvironment. Finally, it would be fascinating to integrate ‘omics approaches with radiomics (quantitative information from digital images) (Pinker et al., 2018) and with imaging-based mass spectrometry that is rapidly changing the field of spatial proteomics (Keren et al., 2018) to guide patient-specific therapy or patient stratification.
This requires collaboration between cancer models with machine learning and network science principles of available data, for instance combining linear mathematical statistical methods to analyze and interpret the vast quantity of 'omics approaches requires powerful computational and an indispensable tool in translational studies. Integration of traditional models in breast cancer research, but also (Huang K. L. et al., 2017).

Transcriptomics and proteogenomics in PDXs have finally helped to profile gene/protein expression to identify novel targets. Genes and their contribution to metastases (Yang et al., 2017) derived cell lines allowed identification of differentially regulated drivers of mesenchymal-to-epithelial transition in 2D culture (Bhatia et al., 2019). Transcriptomics in GEMM and SMM-dysregulated genes and their contribution to metastases (Yang et al., 2017) Transcriptomics and proteogenomics in PDXs have finally helped to profile gene/protein expression to identify novel targets (Huang K. L. et al., 2017).

'Omics technologies have not only improved the power of traditional models in breast cancer research, but also revolutionized the analysis of patient samples, making them an indispensable tool in translational studies. Integration of 'omics approaches requires powerful computational and statistical methods to analyze and interpret the vast quantity of available data, for instance combining linear mathematical models with machine learning and network science principles (Manem et al., 2018). This requires collaboration between cancer scientists, computational biologists and medical statisticians to create robust methods to gain insights into cancer biology and to inform clinical trials and personalized therapeutic regimes.

### Table 2 | A selection of 'omics data repositories built for data sharing and to support research questions (Bamford et al., 2004; Fontaine et al., 2011; Omenn, 2014; Speake et al., 2015; Tomczak et al., 2015; Clough and Barrett, 2016; Rudnick et al., 2016; Chou et al., 2019; Tate et al., 2019).

| Database | 'Omics data | Additional information | References |
|----------|-------------|------------------------|------------|
| Catalogue of Somatic Mutations in Cancer (COSMIC) | × | COSMIC contains data from over 13 million tumor samples, identifying 6 million coding mutations and over 19 million non-coding mutations. This resource collates all genes implicated in cancer through somatic mutation, of which 719 are currently listed. | Bamford et al., 2004; Tate et al., 2019 |
| The Cancer Genome Atlas (TCGA) | × | TCGA contains multi omic data for 30 different tumor types. In regards to breast cancer, it has enabled confirmation of the existence of the four main breast cancer subtypes, it has identified several novel breast cancer drivers and it has identified potentially druggable novel targets. | Tomczak et al., 2015 |
| Clinical Proteomic Tumor Analysis Consortium (CPTAC) | × | CPTAC contains mass spectrometry-based proteomic analysis of tumors from TCGA. The aim of CPTAC is to create a proteogenomic resource where dysregulated proteins and phosphorylation sites can be identified and potentially connected to genomic alterations. | Rudnick et al., 2016 |
| Proteomics Identification Database (PRIDE) | × | PRIDE aims to be a resource for open access sharing of mass spectrometry data, not just across cancer. They currently have over 9200 datasets available, including 297 breast cancer datasets. | Jones et al., 2006 |
| GENIE | × | GENIE combines genomic and clinical data in an attempt to associate genomic alterations with phenotypic changes | Fontaine et al., 2011 |
| GXB | × | GXB compiles immunological transcriptomic data | Speake et al., 2015 |
| Genomic Expression Omnibus (GEO) | × | GEO is a database of transcriptomic and epigenomic data | Clough and Barrett, 2016 |
| Human Proteome Organization (HUPO) | × | The human proteome project, run by HUPO aims to identify all the proteins in the human proteome and to begin to assess their functionalities and interactions | Omenn, 2014 |
| Transcriptome Alterations in Cancer Omnibus (TACCO) | × | TACCO is a resource for identifying differentially regulated transcripts within different cancer types and combining these with survival data to determine prognosis based on gene expression profiles | Chou et al., 2019 |

G, genomics; T, transcriptomics; P, proteomics; M, metabolomics; E, epigenomics.

**'OMICS APPROACHES APPLIED TO EXISTING BREAST CANCER MODELS**

Integrating 'omics approaches with traditional methods has already helped underline the validity of some of the models, for example, highlighting that omics profiles are maintained in PDX models through multiple passages (Zhang et al., 2013). Multomics technologies have also facilitated novel discoveries in existing models (Chakraborty et al., 2018). A combination of genomics, transcriptomics and proteomics has elucidated drivers of mesenchymal-to-epithelial transition in 2D culture (Bhatia et al., 2019). Transcriptomics in GEMM and SMM-derived cell lines allowed identification of differentially regulated genes and their contribution to metastases (Yang et al., 2017). Transcriptomics and proteogenomics in PDXs have finally helped to profile gene/protein expression to identify novel targets (Huang K. L. et al., 2017).

'Omics technologies have not only improved the power of traditional models in breast cancer research, but also revolutionized the analysis of patient samples, making them an indispensable tool in translational studies. Integration of 'omics approaches requires powerful computational and statistical methods to analyze and interpret the vast quantity of available data, for instance combining linear mathematical models with machine learning and network science principles (Manem et al., 2018). This requires collaboration between cancer scientists, computational biologists and medical statisticians to create robust methods to gain insights into cancer biology and to inform clinical trials and personalized therapeutic regimes.

**CONCLUSION AND PERSPECTIVES**

With 'omics technologies applied to patient samples becoming robust, our understanding of the mechanisms driving breast cancer and the discovery of novel biomarkers and therapeutic targets have improved significantly over the last few years (Chakraborty et al., 2018; Manem et al., 2018). For instance, the use of molecular assays, including OncotypeDx and MammaPrint in the clinic is based on advancements in genomic technologies (Gupta et al., 2015; Vieira and Schmitt, 2018). Transparent sharing of 'omics data in databases like COSMIC (Forbes et al., 2015; Vieira and Schmitt, 2018). PRIDE (Jones et al., 2006) and others (Table 2) will allow unbiased analysis of available data by different groups to find previously unnoticed potential genes or proteins of interest as biomarkers or therapeutic targets.

The implementation of 'omics approaches in clinical practice will allow analysis of changes in patients at a global level by improving diagnosis and choice of therapeutic plan so far based on a few markers. We predict that 'omics technologies-guided biomarker identification will allow early tumor detection so that treatments can start earlier and that the identification of novel
targets will decrease reliance on non-targeted therapies, thus improving the quality of life for breast cancer patients.

**AUTHOR CONTRIBUTIONS**

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

**FUNDING**

Research in the lab of CF was supported by Wellcome Trust (Sir Henry Dale Fellowship grant number 107636/Z/15/Z) and by the Biotechnology and Biological Sciences Research Council (BBSRC responsive mode project grant number BB/R015864/1). JP was supported by R-UK Non-Clinical Training Award – 2018 grant number A27445.

**ACKNOWLEDGMENTS**

We thank all members of the Francavilla lab and Dr. Bruno Simoes, Prof. Robert Clarke (The University of Manchester) and Dr. Ciara O’Brien (The Christie Hospital NHS Foundation Trust and The University of Manchester) for helpful discussion and for reading the manuscript. We also thank members of the Bio-MS facility, The University of Manchester. We apologize to authors whose work could not be cited due to space limitations.

**REFERENCES**

Ashton-Prolla, P., Goldim, J. R., Vairo, F. P., da Silveira Matte, U., and Sequeiros, J. (2015). Genomic analysis in the clinic: benefits and challenges for health care professionals and patients in Brazil. *J. Community Genet.* 6, 275–283. doi: 10.1007/s12687-015-0238-0

Azizi, E., Carr, A. J., Plitas, G., Cornish, A. E., Konopacki, C., Prabhakaran, S., et al. (2018). Single-cell map of diverse immune phenotypes in the breast tumor microenvironment. *Cell* 174, 1293.e36–1308.e36. doi: 10.1016/j.cell.2018.05.060

Bamford, S., Dawson, E., Forbes, S., Clements, J., Pettett, R., Dogan, A., et al. (2004). The COSMIC (catalogue of somatic mutations in cancer) database and website. *Br. J. Cancer* 91, 355–361. doi: 10.1038/sj.bjc.6601894

Bartsch, R., Wenzel, C., Zielinski, C. C., and Steger, G. G. (2007). HER-2-positive breast cancer: hope beyond trastuzumab. *Biodrugs* 21, 69–77. doi: 10.2165/00063030-200721020-00001

Beaver, J. A., and Park, B. H. (2012). The BOLERO-2 trial: the addition of everolimus to exemestane in the treatment of postmenopausal hormone receptor-positive advanced breast cancer. *Future Oncol.* 8, 651–657. doi: 10.2217/fon.12.49

Bergom, C., West, C. M., Higginson, D. S., Abazeez, M. E., Arun, B., Benzten, S. M., et al. (2019). The implications of genetic testing on radiotherapy decisions: a guide for radiation oncologists. *Int. J. Radiat. Oncol. Biol. Phys.* 105, 698–712.

Bertucci, F., Ng, C. K. Y., Patsouris, A., Droin, N., Fuksakakis, T., Clements, M., Adolfsion, J., et al. (2014). Time-dependent risk of developing distant metastasis in breast cancer patients according to treatment, age and tumour characteristics. *Br. J. Cancer* 110, 1378–1384. doi: 10.1038/bjc.2014.5

Crujeiras, A. B., Diaz-Lagaras, A., Stefansson, O. A., Macias-Gonzalez, M., Sandoval, J., Cueva, J., et al. (2017). Obesity and menopause modify the epigenomic profile of breast cancer. *Endocr. Relat. Cancer* 24, 351–363. doi: 10.1530/ERC-16-0565

Curts, C., Shah, S. P., Chin, S. F., Tarushvili, G., Rueda, O. M., Dunning, M. J., et al. (2012). The genomic and transcriptomic architecture of 2,800 breast tumours reveals novel subgroups. *Nature* 486, 346–352. doi: 10.1038/nature10983

Dai, X., Cheng, H., Bai, Z., and Li, J. (2017). Breast cancer cell line classification and its relevance with breast tumour subtyping. *J. Cancer* 8, 3131–3141. doi: 10.7150/jca.18457

Davalos, V., Martinez-Cardus, A., and Esteller, M. (2017). The epigenomic revolution in breast cancer: from single-gene to genome-wide next-generation approaches. *Am. J. Pathol.* 187, 2163–2174. doi: 10.1016/j.ajpath.2017.07.002

DeRose, Y. S., Wang, G., Lin, Y. C., Bernard, P. S., Buys, S. S., Ebbert, M. T., et al. (2011). Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. *Nat. Med.* 17, 1514–1520. doi: 10.1038/nm.2454

Djomehri, S. I., Burman, B., Gonzalez, M. E., Takayama, S., and Kleer, C. G. (2019). A reproducible scaffold-free 3D organoid model to study neoplastic progression in breast cancer. *J. Cell Commun. Signal.* 13, 129–143. doi: 10.1007/s12079-018-0498-7

Dobrolecki, L. E., Airhart, S. D., Alferrez, D. G., Aparicio, S., Behbod, F., Bentires-Alj, M., et al. (2016). Patient-derived xenograft (PDX) models in basic and translational breast cancer research. *Cancer Metastasis Rev.* 35, 547–573. doi: 10.1007/s10555-016-9653-x

Doll, S., Gnäd, F., and Mann, M. (2019). The case for proteomics and phospho-proteomics in personalized cancer medicine. *Proteomics Clin. Appl.* 13, e1800113. doi: 10.1002/prca.201800113

Dutta, D., Heo, I., and Clevers, H. (2017). Disease modeling in stem cell-derived 3D organoid systems. *Trends Mol. Med.* 23, 393–410. doi: 10.1016/j.molmed.2017.02.007

Elenbaas, B., Spirio, L., Koerner, F., Fleming, M. D., Zimonjic, D. B., Donaher, J. L., et al. (2001). Human breast cancer cells generated by oncogenic transformation of primary mammary epithelial cells. *Genes Dev.* 15, 50–65. doi: 10.1101/gad.828901
Evans, D. G., Shenton, A., Woodward, E., Laloo, F., Howell, A., and Maher, E. R. (2008). Penetration estimates for BRCA1 and BRCA2 based on genetic testing in a Clinical Cancer Genetics service setting: risks of breast/ovarian cancer quoted should reflect the cancer burden in the family. *BMC Cancer* 8:155. doi: 10.1186/1471-2407-8-155

Falahatkar, S., Navaneethan, T. D., Pe, P., and Borgo, A. (2017). Breast cancer survival by molecular subtype: a population-based analysis of cancer registry data. *CMAJ Open* 5, E734–E739. doi: 10.9778/cmajopen.20170030

Fatehullah, A., Tan, S. H., and Barker, N. (2016). Organoids as an in vitro model of human development and disease. *Nat. Cell Biol.* 18, 246–254. doi: 10.1038/ncb3312

Fisher, B., Anderson, S., Bryant, J., Margolese, R. G., Deutsch, M., Fisher, E. R., et al. (2002). Twenty-year follow-up of a randomized trial comparing total mastectomy, lumpectomy, and lumpectomy plus irradiation for the treatment of invasive breast cancer. *N. Engl. J. Med.* 347, 1233–1241. doi: 10.1056/nejmoa021255

Fontaine, J. F., Priller, F., Barbosa-Silva, A., and Andrade-Navarro, M. A. (2011). Genome- and protein-expression studies and miRNAs and genetically based resistance. *Int. J. Mol. Sci.* 14, 108–145. doi: 10.3390/ijms14010108

Garcia-Becerrra, R., Santos, N., Diaz, L., and Camacho, J. (2012). Mechanisms of for breast cancer. *Ann. Surg. Oncol.* 19, 646–674. doi: 10.1245/s10434-011-1721-9

Huang, F. K., Zhang, G., Lawlor, K., Nazarian, A., Philip, J., Tempst, P., et al. (2017). Deep coverage of global protein expression and phosphorylation in breast tumor cell lines using TMT 10-plex isobaric labeling. *J. Proteome Res.* 16, 1121–1132. doi: 10.1021/acs.jproteome.6b00374

Huang, K. L., Li, S., Mertins, P., Cao, S., Gunawardena, H. P., Ruggles, K. V., et al. (2017). Proteogenomic integration reveals therapeutic targets in breast cancer xenografts. *Nat. Commun.* 8:14864.

Imamura, Y., Mukohara, T., Shimono, Y., Funakoshi, Y., Chayahara, N., Toyoda, M., et al. (2015). Comparison of 2D- and 3D-culture models as drug-testing platforms in breast cancer. *Oncol. Rep.* 33, 1837–1843. doi: 10.3839/or.2015.3767

Iqbal, N., and Iqbal, N. (2014). Human epithelial growth factor receptor 2 (HER2) in cancers: overexpression and therapeutic implications. *Mol. Biol. Int.* 2014:527848. doi: 10.1155/2014/527848

Jasbi, P., Wang, D., Cheng, S. L., Fei, Q., Cui, J. Y., Liu, L., et al. (2019). Breast cancer detection using targeted plasma metabolomics. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 1175, 23–37. doi: 10.1016/j.jchromb.2018.11.029

Jaspers, E. J., Kersbergen, A., Boon, U., Sol, W., van Deemter, L., Zander, S. A., et al. (2013). Loss of 33BP1 causes PARP inhibitor resistance in Brca1-mutated mouse mammary tumors. *Cancer Discov.* 3, 68–81. doi: 10.1158/2159-2890.CD-12-0049

Jerby, L., Wolf, L., Denkert, C., Stein, G. Y., Hilvo, M., Oresic, M., et al. (2012). Metabolic associations of reduced proliferation and oxidative stress in advanced breast cancer. *Cancer Res.* 72, 5712–5720. doi: 10.1158/0008-5472.CAN-12-2215

Jiang, Y. Z., Ma, D., Suo, C., Shi, J., Xue, M., Hu, X., et al. (2019). Genomic and transcriptomic landscape of triple-negative breast cancers: subtypes and treatment strategies. *Cancer Cell* 35, 428.e5–440.e5. doi: 10.1016/j.ccell.2019.02.001

Johansson, H. J., Socciarelli, F., Vacanti, N. M., Haugen, M. H., Zhu, Y., Siavelis, I., et al. (2019). Breast cancer quantitative proteome and proteogenomic landscape. *Nat. Commun.* 10:1600. doi: 10.1038/s41467-019-09018-y

Jones, P., Coté, R. G., Martens, L., Quinn, A. F., Taylor, C. F., Derache, W., et al. (2006). PRIDE: a public repository of protein and peptide identifications for the proteomics community. *Nucleic Acids Res.* 34, D659–D663.

Keren, L., Bosse, M., Marquez, D., Angoštari, R., Jain, S., Varma, S., et al. (2018). A structured tumor-immune microenvironment in triple negative breast cancer revealed by multiplexed ion beam imaging. *Cell* 174, 1373.e19–1387.e19. doi: 10.1016/j.cell.2018.08.039

Kontani, K., Hashimoto, S., Murawaza, C., Norimura, S., Tanaka, H., Ohtani, M., et al. (2014). Factors responsible for long-term survival in metastatic breast cancer. *World J. Surg. Oncol.* 12:344. doi: 10.1186/1748-7925-12-344

Kornblum, N., Zhao, F., Manola, J., Klein, P., Ramaswamy, B., Bruفزky, A., et al. (2018). Randomized phase II trial of fulvestrant plus everolimus or placebo in postmenopausal women with hormone receptor-positive, human epidermal growth factor receptor 2-negative metastatic breast cancer resistant to aromatase inhibitor therapy: results of PReT002. *J. Clin. Oncol.* 36, 1556–1563. doi: 10.1200/JCO.2017.76.9331

Kus, T., Aktas, G., Kalender, M. E., Demiryurek, A. T., Ulasli, M., Oztuzcu, S., et al. (2016). Polymorphism of CYP3A4 and ABCB1 genes increase the risk of neuropathy in breast cancer patients treated with paclitaxel and docetaxel. *Oncol. Targets Ther.* 9, 5073–5080. doi: 10.2147/OTT.S106574

Larramendy, M. L., Lushnikova, T., Bjorkqvist, A. M., Wistuba, I. I., Virmani, K., et al. (2019). Breast cancer quantitative proteome and proteogenomic landscape. *Cancer Discov.* 9, 176–198. doi: 10.1158/2159-8290.CD-18-2032

Larramendy, M. L., Lushnikova, T., Björkqvist, A. M., Wistuba, I. I., Virmani, K., et al. (2019). Breast cancer quantitative proteome and proteogenomic landscape. *Cancer Discov.* 9, 176–198. doi: 10.1158/2159-8290.CD-18-2032

Leelatan, N., Doxie, D. B., Greenplate, A. R., Mobley, B. C., Lehman, J. M., Sinnaeve, J., et al. (2017). Single cell analysis of human tissues and solid tumors. *Sci. Transl. Med.* 9, 119, 359–371. doi: 10.1126/scitranslmed.aab2581

Legrande, C., Gooden, G. C., Johnson, K., Martinez, R. A., Liang, W. S., and Salhia, B. (2015). Whole-genome bisulfite sequencing of cell-free DNA identifies
signature associated with metastatic breast cancer. *Clin. Epigenet.* 7:100. doi: 10.1186/s13148-015-0135-8

Lehmann, B. D., Jovanovic, B., Chen, X., Estrada, M. V., Johnson, K. N., Shyr, Y., et al. (2016). Refinement of triple-negative breast cancer molecular subtypes: implications for neoadjuvant chemotherapy selection. *PLoS One* 11:e0157368. doi: 10.1371/journal.pone.0157368

Li, J., Zhang, Z., Rosenzweig, J., Wang, Y. Y., and Chan, D. W. (2002). Proteomics and bioinformatics approaches for identification of serum biomarkers to detect breast cancer. *Clin. Chem.* 48, 1296–1304.

Linnarsson, S., and Teichmann, S. A. (2016). Single-cell genomics: coming of age. *Genome Biol.* 17:97.

Lisa, M., Cifkova, E., Khalikova, M., Ovcakova, M., and Holcapek, M. (2017). Lipidomic analysis of biological samples: Comparison of liquid chromatography, supercritical fluid chromatography and direct infusion mass spectrometry methods. *J. Chromatogr. A* 1525, 96–108. doi: 10.1016/j.chroma.2017.10.022

Liu, K., Newbury, P. A., Glicksberg, B. S., Zeng, W. Z. D., Paithankar, S., Andrechek, E. R., et al. (2017). High-throughput genomics and clinical outcome in hard-to-treat breast cancer. *Nature* 543, 55–62. doi: 10.1038/nature17676

Omenn, G. S. (2014). The strategy, organization, and progress of the HuPO human proteome project. *J. Proteomics* 100, 3–7. doi: 10.1016/j.jprot.2013.10.012

Park, S. T., and Kim, J. (2016). Trends in Next-generation sequencing and a new Era for whole genome sequencing. *Int. Neurolur. J.* 20, S76–S83.

Pernas, S., Tolaney, S. M., Winer, E. P., and Goel, S. (2018). CDK4/6 inhibition in breast cancer: current practice and future directions. *Ther. Adv. Med. Oncol.* 10:R125. doi: 10.1186/gb-2013-14-11-r125

Pierobon, M., Petricoin, E. F., and Wulkhuhe, J. D. (2018). Phosphoprotein-based drug target activation mapping for precision oncology: a view to the future. *Expert Rev. Proteom.* 15, 851–853. doi: 10.1080/14789450.2018.1537109

Pinker, K., Chin, J., Melsaether, A. N., Morris, E. A., and Moy, L. (2018). Precision medicine and radiogenomics in breast cancer: new approaches toward diagnosis and treatment. *Radiology* 287, 732–747. doi: 10.1148/radiol.2018172171

Playlon, M. C., Ziegler, R. G., Sampson, J. N., Stolzenberg-Solomon, R., Thompson, H. J., Irwin, M. L., et al. (2017). Nutritional metabolomics and breast cancer risk in a prospective study. *Am. J. Clin. Nutr.* 106, 637–649. doi: 10.1093/ajcn/nrx3920

Manem, V. S. K., Salgado, R., Attifmos, P., Sotiriou, C., and Halbe-Kains, B. (2018). Network science in clinical trials: A patient-centered approach. *Semin. Cancer Biol.* 52, 135–150. doi: 10.1016/j.semcancer.2017.12.006

Mardamshina, M., and Geiger, T. (2017). Next-generation proteomics and its application to clinical breast cancer research. *Am. J. Pathol.* 187, 2175–2184. doi: 10.1016/j.ajpath.2017.07.063

Marx, V. (2019). A dream of single-cell proteomics. *Nat. Methods* 16, 809–812. doi: 10.1038/s41592-019-0540-6

Massard, C., Michiels, S., Ferte, C., Le Deley, M. C., Lacroix, L., Hollebecque, A., et al. (2017). High-throughput genomics and clinical outcome in hard-to-treat advanced cancers: results of the MOSCATO 01 trial. *Cancer Discov.* 7, 586–595. doi: 10.1158/2159-8290.CD-16-1396

Masuda, H., Zhang, D., Bartholomeusz, C., Doihara, H., Hortobagyi, G. N., and Ueno, N. T. (2012). Role of epidermal growth factor receptor in breast cancer. *Breast Cancer Res. Treat.* 136, 331–345.

McCann, K. E., and Hurvitz, S. A. (2018). Advances in the use of PARP inhibitor Masuda, H., Zhang, D., Bartholomeusz, C., Doihara, H., Hortobagyi, G. N., and Massard, C., Michiels, S., Ferte, C., Le Deley, M. C., Lacroix, L., Hollebecque, A., et al. (2016). Transproteomics connects somatic mutations to signalling in breast cancer. *Nature* 534, 55–62. doi: 10.1038/nature18003

Michailidou, K., Lindström, S., Dennis, J., Beesly, J., Síia, S., Kar, S., et al. (2017). Association analysis identifies 65 new breast cancer risk loci. *Nature* 551, 92–94. doi: 10.1038/nature22484

Milioli, H. H., Titshchenko, I., Riveros, C., Berrera, R., and Moscato, P. (2017). Basal-like breast cancer: molecular profiles, clinical features and survival outcomes. *BMC Med. Genom.* 10:19. doi: 10.1186/s12920-017-0250-9

Minn, A. J., Kang, Y., Serganova, I., Gupta, G. P., Giri, D. B., Dubrovin, M., et al. (2005). Distinct organ-specific metastatic potential of individual breast cancer cells and primary tumors. *J. Clin. Investig.* 115, 44–55. doi: 10.1172/jci22330

Mohammed, H., Russell, I. A., Stark, R., Rueda, O. M., Hickey, T. E., Tarulli, G. A., et al. (2015). Progestosterone receptor modulates E-box action in breast cancer. *Nat. 523, 313–317.

Molyneux, G., Geyer, F. C., Magnay, F. A., McCarthy, A., Kendrick, H., Natrajan, R., et al. (2010). BRCA1 basal-like breast cancers originate from luminal epithelial progenitors and not from basal stem cells. *Cell Stem Cell* 7, 403–417. doi: 10.1016/j.stem.2010.07.010

Nair, N. U., Das, A., Rokgoti, V. M., Fokkelman, M., Marcotte, R., de Jong, C. G., et al. (2019). Migration rather than proliferation transcriptional signatures are strongly associated with breast cancer patient survival. *Sci. Rep.* 9:10989. doi: 10.1038/s41598-019-47440-w

Neve, R. M., Chin, K., Fridlyand, J., Yeh, J., Baehner, F. L., Fevr, T., et al. (2006). A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. *Cell Cancer* 10, 515–527. doi: 10.1016/j.cccr.2010.08.008

Nik-Zainal, S., Davies, H., Staf, J., Ramakrishna, M., Giodzik, D., Zou, X., et al. (2016). Landscape of somatic mutations in 560 breast cancer whole-genome sequences. *Nature* 534:47. doi: 10.1038/nature17676

Omenn, G. S. (2014). The strategy, organization, and progress of the HuPO human proteome project. *J. Proteomics* 100, 3–7. doi: 10.1016/j.jprot.2013.10.012

Park, S. T., and Kim, J. (2016). Trends in Next-generation sequencing and a new Era for whole genome sequencing. *Int. Neurolur. J.* 20, S76–S83.

Pernas, S., Tolaney, S. M., Winer, E. P., and Goel, S. (2018). CDK4/6 inhibition in breast cancer: current practice and future directions. *Ther. Adv. Med. Oncol.* 10:R125. doi: 10.1186/gb-2013-14-11-r125
Speake, C., Presnell, S., Domico, K., Zeitner, B., Bjork, A., Anderson, D., et al. (2015). An interactive web application for the dissemination of human systems immunology data. J. Transl. Med. 13:196. doi: 10.1186/s12967-015-0541-x
Stender, J. D., Fraas, J., Kimm, B., Chang, K. C., Kraus, W. L., and Katzenellenbogen, B. S. (2007). Estrogen-regulated gene networks in human breast cancer cells: involvement of E2F1 in the regulation of cell proliferation. Mol. Endocrinol. 21, 2112–2123. doi: 10.1210/me.2006-0474
Stephens, P. J., Tarpey, P. S., Davies, H., Van Loo, P., Greenman, C., Wedge, D. C., et al. (2012). The landscape of cancer genes and mutational processes in breast cancer. Nature 486, 400–404. doi: 10.1038/nature11017
Tate, J. G., Bamford, S., Jubb, H. C., Sondka, Z., Bearé, D. M., Bindal, N., et al. (2019). COSMIC: the catalogue of somatic mutations in cancer. Nucleic Acids Res. 47, D941–D947. doi: 10.1093/nar/gky1015
Timms, J. F., White, S. L., O’Hare, M. J., and Waterfield, M. D. (2002). Effects of ErbB-2 overexpression on mitogenic signalling and cell cycle progression in human breast luminal epithelial cells. Oncogene 21, 6573–6586. doi: 10.1038/sj.onc.1205847
Tierir, S. M., Park, J., Preusser, F., Amrhein, L., Gu, Z., Steiger, S., et al. (2019). Pheno-seq - linking visual features and gene expression in 3D cell culture systems. Sci. Rep. 9:12367. doi: 10.1038/s41598-019-48774-1
Tomczak, K., Czerwinska, P., and Wiznowerowicz, M. (2015). The Cancer Genome Atlas (TCGA): an immeasurable source of knowledges. Contemp. Oncol. 19, A68–A77. doi: 10.5114/wo.2014.47136
Tsoutsou, P. G., Vozenin, M. C., Durham, A. D., and Bourhis, J. (2017). How could resistance in breast cancer. Front. Oncol. 7:10259. doi: 10.3389/fonc.2017.01259
Varelija, D., Priedeigk, N., Fagan, A., Purcell, S., Cosgrove, N., O’Halloran, P. J., et al. (2018). Transcriptome characterization of matched primary breast and brain metastatic tumors to detect novel actionable targets. J. Natl. Cancer Inst. 111, 388–398. doi: 10.1093/jnci/dij110
Velasco-Velazquez, M. A., Popov, V. M., Lisanti, M. P., and Pestell, R. G. (2011). The role of breast cancer stem cells in metastasis and therapeutic implications. Am. J. Pathol. 179, 2–11. doi: 10.1016/j.ajpath.2011.03.005
Vieira, A. F., and Schmitt, F. (2018). An update on breast cancer multigene prognostic tests—emergent clinical biomarkers. Front. Med. 5:248. doi: 10.3389/fmed.2018.00248
Vikas, P., Borcherdin, N., and Zhang, W. (2018). The clinical promise of technological advances in phosphoproteomics for cells and tissues. Expert Rev. Proteomics 12, 469–487. doi: 10.1586/14789450.2015.1078730
Vu, T., and Clare, F. X. (2012). Trastuzumab: updated mechanisms of action and resistance in breast cancer. Front. Oncol. 2:62. doi: 10.3389/fonc.2012.00062
Wagner, J., Rapsomaniki, M. A., Chevrier, S., Anzeneder, T., Langwieder, C., Dykgraaf, A., et al. (2019). A single-cell atlas of the tumor and immune ecosystem of human breast cancer. Cell 177, 1330.e18–1345.e18. doi: 10.1016/j.cell.2019.03.005
Wahba, H. A., and El-Hadaad, H. A. (2015). Current approaches in treatment of triple-negative breast cancer. Cancer Biol. Med. 12, 106–116. doi: 10.7497/j.isss.0995-3912.2015.0030
Wakabayashi, M., Yoshihara, H., Masuda, T., Tsukahara, M., Sugiyama, N., and Ishihama, Y. (2014). Phosphoproteome analysis of formalin-fixed and paraffin-embedded tissue sections mounted on microscope slides. J. Proteome Res. 13, 915–924. doi: 10.1021/pr400960r
Weaver, V. M., Howlett, A. R., Langton-Webster, B., Petersen, O. W., and Bissell, M. J. (1995). The development of a functionally relevant cell culture model of progressive human breast cancer. Semin. Cancer Biol. 6, 175–184. doi: 10.1016/s0960-9715.1995.0021
Weeber, F., Oofit, S. N., Dijkstra, K. K., and Voest, E. E. (2017). Tumor organoids as a pre-clinical cancer model for drug discovery. Cell Chem. Biol. 24, 1092–1100. doi: 10.1016/j.chembiol.2017.06.012
Wu, L., Shi, W., Long, J., Guo, X., Michailidou, K., Beesley, J., et al. (2018). A transcriptome-wide association study of 229,000 women identifies new candidate susceptibility genes for breast cancer. Nat. Genet. 50, 968–978. doi: 10.1038/s41588-018-0132-x
Xu, H., Lu, Y., Xi, M., Zhao, W., Song, Y., and Wu, K. (2018). Organoitnology and applications in cancer research. J. Hematol. Oncol. 11:116.
Yang, Y., Yang, H. H., Yu, Y., Watson, P. H., Liu, H., Geiger, T. R., et al. (2017). Immunocompetent mouse allograft models for development of therapies to target breast cancer metastasis. Oncotarget 8, 30621–30643. doi: 10.18632/oncotarget.15695
Yanovich, G., Agmon, H., Harel, M., Sonnenbliek, A., Peretz, T., and Geiger, T. (2018). Clinical proteomics of breast cancer reveals a novel layer of breast cancer classification. Cancer Res. 78, 6001–6010. doi: 10.1158/0008-5472.CAN-18-1079
Yates, L. R., Gerstung, M., Knappskog, S., Desmedt, C., Gundem, G., Van Loo, P., et al. (2015). Subclonal diversification of primary breast cancer revealed by multiregion sequencing. Nat. Med. 21, 751–759. doi: 10.1038/nm.3886
Yates, L. R., Knappskog, S., Wedge, D., Farmery, J. H. R., Gonzalez, S., Martincorena, I., et al. (2017). Genomic evolution of breast cancer metastasis and relapse. Cancer Cell 32, 169.e7–184.e7.
Zagorac, I., Fernandez-Gaitero, S., Penning, R., Post, H., Bueno, M. J., Mouron, A., et al. (2018). An interactive web application for the dissemination of human systems biology data. J. Transl. Med. 16, A68–A77. doi: 10.5114/wo.2014.47136
Zhang, X., Claerhout, S., Prat, A., Dobrolecki, L. E., Petrovic, I., Lai, Q., et al. (2013). A renewable tissue resource of phenotypically stable, biologically and ethnically diverse, patient-derived human breast cancer xenograft models. Cancer Res. 73, 4885–4897. doi: 10.1158/0008-5472.CAN-12-4081
Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Copyright © 2020 Parsons and Francavilla. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.