Review *Clin Ter 2023; 174 Suppl. 2 (6):104-118 doi: 10.7417/CT.2023.2477*

Omics sciences and precision medicine in breast and ovarian cancer

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Abstract

Background: Human breast carcinoma is a complex disease, affecting 1 in 8 women worldwide. The seriousness of the disease increases when the definite cause of the disease remains obscure, thus making prognosis challenging. Researchers are emphasizing on adapting more advanced and targeted therapeutic approaches to address the multifaceted impacts of the disease. Hence, modern multi-omics systems have gained popularity among clinicians, as they offer insights into the genomic, pharmacogenomic, metabolomic, and micro-biomic factors, thus allowing researchers to develop targeted and personalized approaches for breast cancer prevention and early detection, and eventually improving patient outcomes.

Aim. The primary focus of this study is to elucidate, through the integration of multi-omics research findings, the inherent molecular origins of diverse subtypes of breast cancer and to evaluate the effectiveness of these findings in reducing breast cancer-related mortalities.

Methods. Thorough investigation was conducted by reviewing reputable and authoritative medical journals, e-books, and online databases dedicated to cancer research. The Mendelian inheritance in man database (OMIM) was used to scrutinize specific genes and their respective loci associated with the development of different types of breast cancer.

Results. Our present research revealed the holistic picture of sundry molecular, genomic, pharmacogenomic, metabolomic, and micro-biomic features of breast cancer. Such findings, like genetic alterations in highly penetrant genes, plus metabolomic and micro-biomic signatures of breast cancer, unveil valuable insights and show great potential for multi-omics research in breast oncology.

Conclusion. Further research in omics sciences pertaining to breast cancer are at the forefront of shaping precise treatment and bolstering patient survival. ***Clin Ter 2023; 174 Suppl. 2 (6):104-118 doi: 10.7417/CT.2023.2477***

Key words: *breast cancer, precision medicine, genomics, metabolomics, cancer metabolism, biomarker*

Introduction

Breast cancer (BC) has turned out to be one of the most insidious female malignancies worldwide. It is responsible for substantial morbidity and mortality, posing a huge burden on healthcare systems internationally. Recently, GLOBOCAN report (2020) estimated about 2.3 million new breast cancer incidences in a year, which led to 684,996 cancer-related mortalities (about 6.6%) globally (1-3). BC has ranked second among the most frequently diagnosed malignancies, comprising over 11.6% of all female cancers, and placed fifth amongst the most prevalent causes of cancer fatalities (2-4).

Breast cancer is a complex disease that exhibits vast histologic, genetic, and molecular diversity. Even though it is spread worldwide, its occurrence, fatality, and life

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ISSN 1972-6007

expectancy differ greatly in the different areas of the world because of the variable lifestyles of different populations and their genetics, which are known to cause this malady (5). Evidence suggests that BC occurrence is higher in developed countries than in the developing ones (2, 6). However, it has been noted that the rate of BC incidences in high-income countries has met a stabilization and decline, probably due to medical advancement, while its rate in low-income countries has increased (1,3).

BC is most commonly observed in women aged 40-64 years (1, 4). The lifetime risk of females developing breast cancer is approximately 1 in 9 women, with a five-year survival rate as low as <30% (6). Moreover, BC also develops in males, accounting for <1% of all male cancers. This rare cancer type in males is often underestimated and might remain undiagnosed at its early stages because of negligence in checking BC-related risk factors, which are same as female breast cancer: some examples are old age, hormonal dysregulation, radiation exposure, and mutations in BRCA genes (7, 8). Diagnosis is usually made either through screening methods (e.g., X-ray mammography, ultrasonography, etc.), or by detecting particular BC biomarkers at a molecular level, through techniques like immunohistochemistry (IHC), real-time PCR, and nucleic acid hybridization system (9).

Among BC cases, 10% are due to genetic predisposition, while other risk factors include age, environment, obesity, unhealthy diet, use of alcohols & contraceptives, and hormone replacement therapy; the majority of which are potentially modifiable (4, 5). Most susceptible genes responsible for causing breast cancer (BC) also cause ovarian cancer (OC), which commonly leads to hereditary breast-ovarian cancer (HOBC) syndromes (10). BRCA1 and BRCA2 genes have been identified as the mostly examined pathogenic variant genes associated with high risk of breast and ovarian cancer (11). However, there are some non-BRCA genes that are known to increase BC and OC risk (10).

Modern technological advancements in the field of 'omics' studies have undoubtedly helped a lot in clarifying genetic etiology and pathological changes in breast cancer. An extensive availability of multi-omics databases to pile up large-scale genomic, epigenetic, transcriptomic, and proteomic information is crucial for patient stratification, early prognostication of biomarkers and development of potentially targetable precision treatment to upgrade overall survival (12, 13).

Review articles in cancer research provide a comprehensive overview of numerous researches, also aiding in therapeutic planning and clinical management (5). In this review article, we intended to delve deep into the oncology of breast cancer in the context of its genetics, genomics, pharmacogenomics, metabolomics, and micro-biomics research, to elaborate genomic and histopathologic characteristics of BC for the development of meticulous prognosis and therapeutic management in the future.

Histopathologic findings and BC classification

Breast cancers exhibit immense heterogeneity in histopathological features and oncogenesis, posing great challenges for diagnosis and clinical decision making. Histologically, BC can be identified by uncontrolled cell growth either in the ducts or in the lobules of breasts. Depending on its anatomical origin, breast carcinoma broadly falls into two categories:

1. Invasive ductal carcinoma (IDC): the most common type of BC (accounting for about 80% of all cases). It begins in the cells that line the milk ducts and grows into the surrounding breast tissues (14);
2. Invasive lobular carcinoma (ILC): the less common type of BC (accounting for about 10-15% of all cases). It begins in the cells of milk-producing glands, or lobules (14).

In addition, the classification of invasive BC is also done through the 3-tier (low grade, intermediate grade, and high-grade) system, which comprehends the microscopic appearance of tumors (12). However, the extent and severity of BC is determined by a definite staging system, which is a different concept from grading. Staging refers to the process of determining the tumor potential to metastasize in stages from 0-IV. It is based on anatomic findings like the percentage of tumor (T) in breast tissues, the degree of lymph nodes involvement (N), and mitotic rate or metastasis (M). Both systems are heavily utilized in clinical practice and important to determine the best course of treatment (12, 14). A widely used method of breast cancer staging is the Nottingham Prognostic Index (NPI), that combines the scores of different histologic and molecular features to state the prognosis (3, 14).

Molecular features and subtyping of BC

An updated prognostic system of breast cancer staging has been published by the American Joint Committee on Cancers (AJCC) 8th edition in 2018, which also acknowledges molecular features of BC in addition to histological features (3).

On the basis of mRNA gene expression levels, BC has been divided into four intrinsic or molecular subtypes, named as luminal A, luminal B, HER2-enriched, and basal-like or triple-negative BC (3, 12). The additional features included in this classification are:

- Estrogen receptor (ER) expression levels,
- Progesterone receptor (PR) expression, and
- Oncogenic Human Epidermal growth factor Receptor-2 (HER2) overexpression (12).

It is worth mentioning that ER and PR are the receptors that cause the stimulation of cellular growth in normal and neoplastic conditions, and they are overexpressed in nearly 75% of BC cases. HER2 is found overexpressed in 15% of BC cases, and is characterized by aggressive invasion and poor prognosis. ER, PR, and HER2 overexpression serves as an important biomarker and is predictive of hormonal and anti-HER2 targeted therapy. Sometimes, about 10-15% of BCs are not diagnosed with either of three biomarkers and are thus called triple negative breast cancers (TNBC) (12).

This molecular subtyping of BC is now performed through the PAM50 gene expression test, which examines the activity of 50 different signature genes (15). It is able to classify BC into the abovementioned intrinsic subtypes with >90% accuracy. PAM50 uses a technology called quantita-

tive reverse transcription polymerase chain reaction (qRT-PCR) to measure the expression levels of these genes in a tumor sample, to help in the prognosis and guide treatment decisions in BC patients (3, 15).

Genetic basis of breast cancer

About 5-20% cases of breast and ovarian cancers are due to hereditary defects in pathogenic variant genes (11, 16). Studying genetics of breast cancer is thus essential to gain a better understanding of the genetic changes that lead to this malignancy, and to get help in designing targeted therapies and improving early detection of BC in high-risk individuals.

BRCA1 and BRCA2 are two well-known pathogenic variant genes predisposing individuals to breast-ovarian cancers. There are also certain non-BRCA genes and other hereditary predisposition syndromes leading to breast-ovarian cancers. Some well-recognized inherited syndromes include Lynch syndrome, NTHL1 tumor syndrome, MUTYH-associated polyposis, familial adenomatous polyposis, and hereditary breast and ovarian syndromes. Detecting these hereditary syndromes through genetic testing can be helpful in the identification of high-risk individuals, early discovery of breast and ovarian cancer probability, and timely personalized treatment (11).

Genetic testing generates a great quantity of data and its execution requires genetic counseling. Therefore, some mathematical models have been generated, which can predict the probability of carrying pathogenic variant genes and the breast-ovarian cancer risks by using the description of family history. One of such susceptibility models is BOADICEA (Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm), formulated by Antoniou et al. in 2004. This model predicts the susceptibility to breast cancer by combining the multiplicative effect of mutations in BRCA1/2 genes along with multiple other genes. Risk predictions by this model are nearly the same as those observed through population-based studies (17).

Another similar approach is the Mendelian Randomization (MR) model, which is a statistical technique used to investigate the causal relationship between a specific risk factor and the disease or health outcome. Based on Mendelian genetics, MR has been also used to investigate the risk factors of breast cancer. For instance, a study by Guo et al. used the MR tool to identify the causal relationship between body mass index and breast cancer risk. Genetic variants associated with BMI were examined as instrumental variables to estimate the causal effects. The study found that a higher BMI was causally associated with an increased risk of breast cancer (18).

One more similar study conducted by Vanhevel et al., utilized the MR approach to investigate the causal relationship between vitamin D levels and BC risk. The study used certain genetic variants responsible for vitamin D levels to estimate the causal effect of vitamin D on breast cancer risk. Results of the study showed that higher genetically predic-

Table 1. Selected inherited genetic alterations with genes and genetic syndromes that cause breast and ovarian cancers (3, 16, 20).

Inherited syndromes	MIMs of syndrome phenotypes	Genes	Cytogenetic locations	Gene OMIMs	Lifetime cancer risk	Inheritance
Hereditary Breast-ovarian cancer (HBOC) syndrome, familial, 1	604370	BRCA1	17q21.31	113705	Breast, ovarian, prostate	AD
Hereditary Breast-ovarian cancer (HBOC) syndrome, familial, 2	612555	BRCA2	13q13.1	600185	Breast, ovarian, prostate, melanoma, pancreatic, gall bladder	AD
Fanconi anemia, complementation group J	609054	BRIP1	17q23.2	605882	Ovarian	AR
Hereditary Diffuse Gastric and lobular breast Cancer syndrome	137215	CDH1	16q22.1	192090	Gastric, Lobular breast	AD
N/A	Nil	CHEK2	22q12.1	604373	Breast	AR
Fanconi anemia, complementation group N	610832	PALB2	16p12.2	610355	Breast, ovarian	AR
Cowden syndrome, Lhermitte-Duclos disease	158350	PTEN	10q23.31	601728	Breast	AD
BROVCA3	613399	RAD51C	17q22	602774	Ovarian	
BROVCA4	614291	RAD51D	17q12	602954	Ovarian	
Li-Fraumeni syndrome	151623	TP53	17p13.1	191170	Breast, soft tissue sarcoma, brain, osteosarcoma	AD
Lynch syndrome 4	614337	PMS2	7p22.1	600259	Ovarian	

Abbreviations: *AD, autosomal dominant; AR, autosomal recessive; MIM, mendelian inheritance in man, BROVCA, breast-ovarian cancer.*

ted vitamin D levels possess anti-cancer activity and were associated with a lower risk of breast cancer (19). In short, MR has the potential to provide valuable insights into the underlying causes of breast cancer and help in the development of new prevention and treatment strategies.

It has been proved that about 5-20% breast and ovarian carcinomas are due to genetic abnormalities. Researchers have attempted to explore the exact polygenic genetic architecture responsible to cause BC: in 2019, Lu and colleagues obtained a set of 11 susceptibility genes potentially responsible to cause BC and OV through large-scale exome sequencing (16). Here, we are enlisting these predisposition genes (**Table 1**) with MIM (Mendelian inheritance in man) status, whose variants are responsible for causing breast cancers.

Multigene panel testing identifies pathogenic variants that harbor the risk of breast and ovarian cancers (16). BRCA1, BRCA2, TP53, PTEN, STK11, and CDH1 are the genes that are considered as highly penetrant genes that predispose to breast-ovarian cancer, while PALB2, BRIP1, CHEK2, and other Fanconi anemia genes are considered as low or moderate penetrant genes that predispose to breast and ovarian cancers. The following paragraphs will give a brief description of some predisposition syndromes that increase BC risks (21).

Hereditary breast and ovarian syndromes

BRCA1 and BRCA2 are high penetrance genes that are known to cause hereditary breast-ovarian cancer syndromes (BROVCA). The gene BRCA1 is located on 17q21.31, that codes for BRCA1 protein. It is a tumor suppressor protein that acts in combination with DNA damage sensors and signal transducers to make a huge protein complex called BRCA1-associated genome surveillance complex, or BASC (21). On the other hand, BRCA2 is located on chromosome 13q13.1 and codes for a nuclear protein responsible for DNA repair by homologous recombination. Mutations in BRCA1/2 lead to the high risk of breast-ovarian cancer in females, along with increased risk of developing BC in males (21).

Pathogenic variants of a single BRCA gene inherited from one of the parents do not always lead to the development of BC, but a second mutation that could affect the pathogenic variant may increase the susceptibility of BC. Therefore, BRCA1/2 mutations possess great prognostic value and remain the focus of genetic testing to predict the risk of BC (14).

Lynch syndrome

Lynch syndrome (LYNCH), also called hereditary non-polyposis colorectal cancer, is an inherited condition that increases the risk of developing several cancers, including ovarian cancer (22). It is an autosomal dominant disorder that can be diagnosed through early onset of colorectal cancer. Lynch syndrome is caused by mutations in the PMS2 gene, located on chromosome 7p22.1, which is a DNA mismatch repair gene. Multiple studies have observed the presentation of colorectal cancers due to monoallelic mutations in PMS2 (23).

NTHL1 tumor syndrome

NTHL1 tumor syndrome refers to a genetic condition, caused by mutation in the NTHL1 gene located on chromosome 16p13.3, responsible for code repair enzymes like DNA N-glycosylases of the endonuclease III family (24). NTHL1 is a DNA repair gene, and its mutation may favor the growth of various cancers, including breast cancers. This inherited condition is also called familial adenomatous polyposis (FAP), and is associated, although rare, with an increased risk of breast cancer (25).

MUTYH-associated polyposis

MUTYH-associated polyposis (MAP) is an inherited syndrome, characterized by multiple polyps in the colon. MUTYH is a base excision repair (BER) gene, located on chromosome 1p34.1, responsible to sidestep DNA damage from methylation, deamination, reactive oxygen species, and hydroxylation. Germline biallelic MUTYH pathogenic variants are correlated with the development of MAP, which markedly increases the chances of colorectal cancer (CRC) development. In addition, the risk for malignancies of the bladder, breast, ovary, and endometrium also increase (26). Identification of MUTYH mutations by genetic testing can predict the risk of suspected cancers and can improve surveillance and intervention strategies to counteract cancer incidences in the future (11).

Li-Fraumeni syndrome

Li-Fraumeni syndrome is an autosomal dominant disorder caused by mutation in TP53 gene, located on chromosome 17p13.1. It encodes for TP53 protein, which is an important tumor suppressor protein that induces cell cycle arrest, apoptosis, and DNA repair mechanisms. TP53 mutation causes great predisposition to various cancers such as brain, sarcoma, and breast cancer. Therefore, due to increased chances of developing BC, individuals with Li-Fraumeni syndrome are suggested to keep regular examination for BC every 6-12 months, with MRI screening annually (21).

Genomic hallmarks of Breast Cancer

Breast carcinogenesis emerges by inherited genetic variations and acquired genomic aberrations that lead to abnormal cellular proliferation. Mutations of specific genes make up the molecular basis of any cancer, however, malignant transformation is a multi-step process that also depends on somatic mutations, copy number aberrations, DNA repair defects, epigenetic changes, and structural rearrangements of chromosomes like deletions, amplifications, inversions, and translocations. Delineating these events is only possible by whole genome sequencing, which is becoming a standard research tool to study breast cancer progression (27). Genomics is a powerful approach to analyze family history of breast cancer in high-risk individuals, who thus may be subjected to prior screening and will be benefitted with early diagnosis and risk assessment of the disease (3,28).

Correlated pathologies, as lymphedema, can also have a genetic cause (29-34)

Whole genome profiling of large cohorts of breast cancer has demonstrated different deviational processes that are distinct in each subtype, termed as genomic mutational signatures (35). These mutational processes include abnormal DNA editing, aberrant replication, base substitution, tandem duplication/deletion, flawed DNA repair, mismatch repair deficiency, and mutations by carcinogen exposures (12). Each particular BC subtype may harbor more than 1 mutational signature, and thus aid in patient stratification.

Genomic hallmarks of breast cancer are crucial to attain a more holistic picture of BC complexities. For this purpose, The Cancer Genome Atlas (TCGA) has used six different domains of information that provide the basis of genomic instability in BC (12). Here we will briefly discuss each domain, to discover breast cancer's genomic diversity.

1. **Distinct mRNA expression of BC subtypes-** mRNA expression microarray is a powerful approach, used to measure the expression levels of thousands of genes simultaneously in a breast cancer tissue. This approach has been used to identify the genes that are differentially expressed in cancerous and normal breast tissues, and to spot the genes responsible for cancer development and progression. Depending upon mRNA expression, BC has been classified into 4 intrinsic subtypes, named luminal A and B, HER2 positive, and basal-like or triple-negative BC (36).
   Each BC subtype showed signature mutations: for example, luminal A and B exhibited mutations in PIK3CA, GATA3 or MAP3K1 genes, while triple-negative BC showed mutations in BRCA1, TP53, and RB1 genes with MYC amplifications (35). This subtyping is very helpful in predicting patient outcomes and their response to specific treatments like hormone therapy or chemotherapy (36).
2. **Differential DNA methylation of specific subtypes**- DNA methylation signatures in BC have valuable diagnostic potential and comprise a robust system to improve disease management. DNA methylome sequencing is performed through a technique called DNA methylation chips, through which we can measure the methylation status of numerous CpG sites across the genome (12).
   DNA methylation is an essential epigenetic mechanism, in which a methyl group is added to the cytosine nucleotide of a CpG dinucleotide. This mechanism is important to regulate gene expression, and its aberration causes gene expression abnormality and cancer progression. For instance, the profiling of a methylome in triple-negative breast cancer showed that differentially methylated regions (DMRs), associated with TNBC, serve as potential biomarkers for this BC subtype (37). Moreover, the association of specific DMRs with different BC subtypes has implications for diagnosis and therapeutic outcomes.
3. **Single nucleotide polymorphism (SNPs)-** SNPs in BC may be germline (inherited) or somatic (acquired) mutations that have potential to improve precision of BC risk prediction. BRCA1/BRCA2 are the most commonly observed germline mutations, which are known as BC-predisposition pathogenic variants. Next generation DNA sequencing has also led to the discovery of additional germline mutations—including BARD1, PALB2, RAD51D, BRIP1, RAD51C, and TP53—that are linked with moderate to high risk of triple-negative BC (38). Currently, the Genome Wide Association Studies (GWAS) database of published SNP-trait associations has identified 182 SNPs linked with BC, which are mentioned in about 53 studies (38).
   Somatic mutations potentially promote BC growth and metastasis, and can be specifically demonstrated in particular subtypes. Notably, a study by TCGA showed that three somatic mutations (TP53, PIK3CA, and GATA3) are common in all BC subtypes, with over 10% occurrence. Whereas, many subtype-associated mutations also exist in GATA3, PIK3CA and MAP3K1 in Luminal A BC (39).
4. **Aberration in microRNA (miRNA) expression-** miRNA are small non-coding RNAs that participate in post-transcriptional gene regulation. Their aberrant expression is known to promote cancer instigation, and it is also associated with breast cancer progression (12, 39). Moreover, the dysregulation of miRNA expression also shows distinct signatures that are used to classify BC subtypes and predict clinical outcomes. For example, a study showed that the downregulation of four miRNAs (miR-221, miR-1305, miR-4708, and RMDN2) in TNBC leads to more aggressive breast tumors, with poor prognosis (40). Similarly, the Luminal A subtype is further divided into two subgroups, depending on the differential expression of miRNAs (12).
5. **Significantly mutated genes (SMGs) and altered DNA copy number (CNAs)-** Whole exome sequencing of plenteous breast tissue samples showed 30,626 somatic mutations, including ample point mutations, few dinucleotide mutations, and abundant insertions/deletions (indels). This greatly helps in identifying frequently mutated genes in breast cancer—including TP53, PIK3CA, GATA3, and MAP3K1—and also driver mutations that promote BC growth and progression (39).
   Mutations like deletions, amplifications, and rearrangements cause changes in the number of copies of DNA segments in the genome, also called copy number alterations (CNAs). Large-scale studies have identified specific CNAs that are associated with breast cancer development and progression. Focal amplification of segments holding PIK3CA, EGFR, FOXA1, and HER2, and focal deletions of segments holding MLL3, PTEN, RB1, and MAP2K4 were found to cause CNAs in breast cancer (39).
   Similarly, a study analyzed genome rearrangements in breast cancer and their association with patient survival. In a large cohort of BC patients, genome-wide copy number alterations were analyzed to

discover certain novel and recurrent CNAs specific to BC subtypes. This copy number profiling has implications for making clinical decisions and targeting specific genome rearrangements in breast cancer (41).

6. **Presence of tumor antigens as protein biomarkers-** Reverse-phase protein array (RPPA) is an antibody-based technique that measures protein expression levels in BC tissues to identify protein biomarkers and drug targets, to provide insights into the molecular mechanisms underlying the disease, and to guide the development of personalized therapies. Protein microarrays have the potential to simultaneously present and assess hundreds of tumor antigens (42). A study used RPPA to screen for autoantibodies in serum samples from BC patients and healthy controls. The results of this study showed high sensitivity and specificity for the detection of early-stage breast cancer (42).

Similarly, on the basis of protein expression, two novel protein-defined subgroups of breast cancer were also made. These are reactive I and reactive II groups, composed primarily of a subset of Luminal A tumors and a mixture of mRNA subtypes. These groups are termed 'active' because of the occurrence of proteins generated in the tumor microenvironment and cancer-activated fibroblasts (39).

An expansion in genomics of the research on breast cancer is inevitable to gain the wide-ranging list of recurrent mutations found in BC. By sequencing tumor genomes at both early and advancing stages, we can pinpoint essential cellular pathways to annotate with pharmacogenomics and to identify potential therapeutic targets (27).

Pharmacogenomics of breast cancer

Selecting a particular drug for a breast cancer patient depends, to some extent, on their genetic makeup: genetic alterations, even of a single nucleotide, noticeably impact the activity and expression of proteins involved in the pharmacokinetics and pharmacodynamics of therapeutic drugs. Therefore, variations in germline DNA are the prime focus of pharmacogenomics, because they can significantly alter drug metabolism and its therapeutic outcomes (43). Ultimately, pharmacogenomic studies are aimed at identifying single nucleotide polymorphisms (SNPs) and other genomic alterations in particular patients, determining targets for selected drugs, and improving their efficacy and safety in clinical settings (43, 44).

The selection of suitable treatment for operable breast cancer involves both local therapies (surgery and radiation) and systemic therapies (by various drugs). Systemic therapy plays a crucial role in upsurging disease-free survival (DFS) by diminishing the tumor potential and controlling micro-metastasis (45). The drugs used for systemic therapy generally fall into three classes: hormonal therapy, targeted therapy, and chemotherapy. These therapeutic drugs can be given alone or in multi-drug regimens. In this review, we will be focusing on pharmacogenomics of drugs employed for breast cancer patients and their clinical applications with probable outcomes.

Stratification of patients for appropriate treatment

Phenotypic subtypes of BC are very important in selecting suitable treatment options (45). For example (44, 46, 47):

- Luminal A- ER/PR +ve – treated with endocrine receptors modulators like tamoxifen or aromatase inhibitors;
- Luminal B- ER/PR +ve – also treated with hormonal therapy;
- Metastatic Luminal BC – treated with selective CDK4/6 inhibitors such as Palbociclib;
- HER2 and TNBC – treated with Antibody–Drug Conjugates (ADCs);
- HER2 enriched- HER2 +ve – treated with anti-HER2 therapies, like trastuzumab;
- TNBC- All receptors negative – treated with chemotherapy.

Principally, the choice of suitable therapeutic strategy for an individual patient is a multi-disciplinary process, and many factors are to be considered. Various factors include age, menopausal status, physical and mental health, and clinical phenotypes such as tumor size, nodal status, invasiveness, expression of hormonal receptors, and the patient's genetic constitution (48). Decisions about systemic therapy are made by predicting drug responses and examining tumor sensitivity to drugs. Also benefits, adverse events, and costs are to be determined. For the decision of timings of systemic therapies, adjuvant (after surgery) and neoadjuvant (before surgery) methods intended to improve DFS are being tailored for each particular patient (45).

Like other cancer patients, BC patients possess two types of genomes. One is their own integral germline genome, and the other is the altered tumor somatic genome (44). The somatic genome is eccentric from their constitutional genome because it harbors inherited genetic alterations in addition to acquired genomic variations, which are either oncogenic 'driver mutations' or established during carcinogenesis as 'passenger mutations'. Somatic or driver mutations surmount principal focus in the quest of targeted therapy, because they are actively involved in tumorigenesis and provide a selective growth advantage to BC. On the other hand, passenger mutations do not confer much growth advantage to breast tumor and gained less attention of researchers because they are not ideal targets to develop precision drugs (35, 44).

These idiosyncratic genomic variations, which are responsible to cause pathogenic event or are influenced by drugs, are designated as genetic biomarkers (44). Genetic alteration of even one nucleotide can result in either lack or deviant enzyme activity, which produces a huge impact on drug response. This way, a single nucleotide polymorphism (SNP) can influence drug toxicity and efficacy in numerous ways (43).

Hormonal therapy

Nearly 80% of BCs are classified as estrogen receptor (ER)-positive, out of which 65% are also progesterone receptor (PR)-positive (43, 49). These receptors are actually

nuclear proteins that regulate the expression of specific genes and predict the patient's sensitivity to endocrine manipulations. Estrogen regulates various cellular activities, including breast tumor cell proliferation. Therefore, hormonal therapy is considered as complementary to surgery in the majority of cases, because it blocks hormonal receptors and inhibits tumor progression.

There are two major types of hormone receptor blockers that are approved for BC treatment, i.e., selective estrogen receptor modulators (SERMs), and the third-generation aromatase inhibitors (AIs). Since estrogen is the major culprit of causing BC in ER +ve patients, estrogen therapy (ET) can have different targets, which are mainly:

- Suppression of the ovaries' function, since they are the main source of estrogen and progesterone hormones;
- Use of selective estrogen receptor modulators (SERMs), which prevent the uptake of estrogen by the receptor;
- Use of selective estrogen receptor downregulators (SERDs), which, like SERMs, impede ER binding to cancer cells, thus reducing their proliferation and survival;
- Inhibition of the key enzyme involved in estrogen biosynthesis (aromatase inhibitors) (49).

All of the above stratagems have different pharmacologic properties, biochemical nature, and molecular organization, but ultimately they all disrupt estrogen signaling. Below we discuss each class of drugs that inhibit proliferation of estrogen-dependent BC cells, but taking different metabolism routes, which is controlled genetically (43).

Ovarian functional suppression (OFS)

Ovarian functional suppression (OFS) is usually achieved either through oophorectomy (surgical removal of one or both ovaries) or by infusion of gonadotropin releasing hormone agonists (GnRHa) (49).

Gonadotropin releasing hormone (GnRH) is an essential hormone for the synthesis of sex hormones. Released from hypothalamus, GnRH stimulates pituitary gland to produce luteinizing hormone (LH) and follicle stimulating hormone (FSH), the two of which cause the gonads to make sex hormones, i.e., testosterone in males, and estrogen and progesterone in females. GnRH agonists have been used to inhibit the levels of sex hormones because, after an initial transient surge in sex hormone levels, they cause a decline in their release. Luckily, GnRH analogs have proved to be much more effective and protracted in producing the same efficacy, without the initial surge. An excellent example of a GnRH analog is degarelix (50).

Selective estrogen receptor modulators (SERMs)

Selective estrogen receptor modulators (SERMs) are nonsteroidal molecules that can bind to ER and exert agonist or antagonist actions. Among them, the most effective and widely used SERM is tamoxifen (TAM), which is considered as standard adjuvant treatment prescribed at initial stages of BC to women over 30 years of age. Its use for five years substantially decreases the risk of recurrence and mortality by BC. Tamoxifen undergoes extensive metabolism in liver through cytochrome p450 enzyme system (CYP450), leading to the production of highly active metabolites that are more dynamic than its original form and show remarkable pharmacological impressions (43).

One of such metabolites is 4-hydroxy tamoxifen, which possesses thirty to hundred-fold greater potency in crushing ER-dependent cell proliferation than tamoxifen Similarly, endoxifen is another metabolite that also effectively inhibits ER activity and ER-positive cell proliferation. Genetic variations in genes that encode enzymes for tamoxifen metabolism have pharmacogenomic importance in predicting BC outcomes (43).

Selective estrogen receptor downregulators (SERDs)

Selective estrogen receptor downregulators (SERDs) are compounds capable of reducing the ER protein level and blocking ER activity, thus also acting as ER antagonists. A well-known ER downregulator is fulvestrant, which binds with ER, thus preventing its dimerization and eventually leading to its degradation.

Fulvestrant is an FDA-approved SERD for BC patients that has greater affinity for ER and causes less adverse effects on endometrial ERs. Particularly, it is useful for patients with advanced BC stages and serves as second-line therapy in TAM-resistant BC patients (49,51).

Aromatase inhibitors (AIs)

Third-generation aromatase inhibitors work by counteracting the key enzyme aromatase, which converts androgens to estrogens. Unlike tamoxifen, AIs are active in their parent form and deactivated by metabolism. Anastrazole, letrozole, and exemestane are few examples of AIs, that are found to block the activity of aromatase enzyme by 96-99%, leading to much lower endogenous estrogen levels than those seen in natural menopause in postmenopausal women (43).

The enzyme aromatase, responsible for synthesizing estrogen, is encoded by gene CYP19A1. Therefore, this gene serves as a target of interest and has considerable pharmacogenomic importance in inhibiting aromatase. SNPs in CYP19A1 have shown improved efficacy of AIs in the neoadjuvant and adjuvant settings (43). A study on AI-treated postmenopausal women in an adjuvant setting showed that two SNPs in the aromatase gene CYP19 caused significant change in aromatase activity after AI therapy.

Targeted therapy

Genetic variations in tumor cells influence drug metabolism, and have been recognized to select suitable therapeutic regimens to reduce resistance. With the advancement of pharmacogenomic approaches in breast oncology, targeted therapies have led to the implementation of novel drug regimens that confer maximum drug efficacy with minimum toxicity (51). Targeted therapies are generally implemented to treat patients who express several distinctive proteins on tumor cell surface, called tumor-associated antigens (TAAs), which promote abnormal growth patterns. For this purpose,

antibodies are mostly used as they function like the human immune system (52).

TAAs have been successfully targeted by novel drugs and bispecific antibodies. Nowadays, the most effective targeted therapy for BC focuses on inhibiting the overexpression of HER2 protein, found on the surface of BC cells. Trastuzumab is the first humanized monoclonal antibody to receive FDA approval against HER2 receptor in breast cancer (51).

Currently, there are seven widely used targeted therapies that effectively block various molecular pathways in BC (52):

1. Afinitor or everolimus: it is an m-TOR inhibitor that obstructs energy supplies of BC cells;
2. Avastin or bevacizumab: it diminishes the formation of new blood vessels and thus blocks the oxygen and nutrient supply of cancer cells;
3. Herceptin or trastuzumab: a monoclonal antibody that binds to HER2 receptor, inhibiting cell proliferation and halting their growth;
4. Kadcyla or T-DM1: it is a combination of Herceptin and emtansine. It transports emtansine chemotherapy to cancer cells;
5. Perjita or pertuzumab: it blocks the growth signals of cancer cells;
6. Tykerb or lapatinib: it is HER2 inhibitor that inhibits cell growth;

In general, TNBC patients respond to a targeted treatment comprising PARP1 inhibitors, taxol derivatives, and anthracycline chemotherapy. The patients who are resistant to anthracycline and taxane drugs may be treated with microtubule-stabilizing agents ixabepilone and capecitabine (52).

Cytotoxic chemotherapy

Evolving from single alkylating agents to multiple chemotherapy regimens, cytotoxic chemotherapy has made substantial progress in treating both advanced and early-stage BC. BC patients' therapeutic index to cytotoxic drug is unique for every patient, and pharmacogenomics (PGx) may play an important role in explaining individual differences of chemotherapeutic outcomes.

Antimetabolites

Capecitabine is an orally administered, third-generation effective drug that is a pyrimidine analog 5-flourouracil (5-FU). It is one of the best treatments for triple-negative BC patients and metastatic BC, where capecitabine has proved to remarkably improve overall survival rate (44). Capecitabine potentially halts tumor growth by inhibiting DNA synthesis in cancerous cells. However, its catabolism-related defects may result in drug accumulation and toxicity. 5-FU prodrug capecitabine is catabolized by enzyme dihydropyrimidine dehydrogenase (DPD), which is coded by gene DPYD. Pharmacogenomics studies revealed that the key enzyme responsible to convert capecitabine into 5-FU may undergo genetic deactivation and lead to drug toxicity (43).

Gemcitabine is an antimetabolite and nucleoside analog that brings about tumor apoptosis by inhibiting DNA replication. It is commonly used in the treatment regimens for metastatic and advanced BC. Gemcitabine-related hematologic toxicity, tumor response and survival rate are variable due to the SNPs variations in genes encoding drug metabolizing enzymes in BC patients (43).

Anti-microtubules

Taxanes are microtubule antagonists, considered as powerful cytotoxic agents, that efficaciously improve overall survival in adjuvant and neo-adjuvant chemotherapies in BC. They include paclitaxel and its semi-synthetic analog docatexel, which target microtubules and inhibit the dynamics of these mitotic spindles. This way, they cause cytotoxicity in tumors by blocking mitosis and triggering cell death in tumor cells (44).

Taxanes are hydroxylated in the hepatic CYP3A4 system. Paclitaxel is further metabolized by CYP2C8, and the genes encoding its metabolism undergo genotypic variations that influence the clearance rate of paclitaxel. Research about paclitaxel-containing regimes in BC patients showed variable treatment response: patients harboring the variant allele of this gene CYP2C8*3 are at increased risk of getting severe peripheral neurotoxicity. This taxane-induced peripheral neuropathy (TIPN) in BC patients can be devastating and treatment-limiting (43). Genes that are linked to cause TIPN serve as pharmacogenomic biomarkers that may alter treatment outcomes.

Anthracyclines

Anthracyclines make up a class of drugs that suppress enzyme topoisomerase II, thus causing apoptosis of tumor cells. Doxorubicin, epirubicin, daunorubicin, and idarubicin are different anthracyclines that are potent anti-tumor chemotherapeutic agents, extensively used in adjuvant and neoadjuvant settings, and showed improved survival rates. Their combination with cyclophosphamide (AC) is the foundation of chemotherapy regimens used to treat BC, which has replaced many other regimens due to its exceptionally favorable outcomes with BC patients (44).

A noteworthy limiting factor in consuming these potential cytotoxic agents is the so-called anthracycline-induced cytotoxicity (AIC), an adverse event that manifests as serious cardiotoxicity, hematological toxicity, gastrointestinal toxicity, and febrile neutropenia. Pharmacogenetic variations in enzymes that code for anthracycline metabolism and transport, and cause oxidative stress have been defined in the literature, but lack substantial evidence for association with AIC in BC. For this purpose, variants of carbonyl reductase (CBR1 and CBR3), a doxorubicin metabolizing enzyme, have been widely studied, but no significant association of genetic variation with AIC has been observed (43).

Cyclophosphamides

Cyclophosphamide is considered as a mainstay in almost all BC chemotherapeutic treatments. It is a DNA alkylating agent, that is a prodrug that undergoes hepatic metabolism,

primarily governed by CYP3A4, CYP2B6, and CYP2C9 genes (43). Cyclophosphamide metabolites exhibit significant variations in different patients' plasma concentrations, predicting that its metabolism is influenced by genetic variations. The clearance of cyclophosphamide is also under genetic control, and genetic variations in its pathway may be suitable pharmacogenetic targets. An enzyme that causes detoxification of cyclophosphamide metabolites is ALDH1A1, which has also been linked with poor prognosis of the basal-like breast cancer subtype (43). Its chronic use may induce cytotoxicity, i.e., hematological toxicity, nausea, vomiting, and reversible alopecia (44).

With the rapid development of pharmacogenomics and bioinformatics, the management of studies on pharmacogenomic biomarkers of BC has become much faster than in the past. Due to the diversity of patient responses to anticancer medications and their narrow therapeutic index, the above-described pharmacogenomic disciplines have scope to provide tailored oncological treatments for breast cancer. While the initial attention was given to protein markers, such as ER and HER2 receptors, a significant number of the latest predictive biomarkers employed to direct treatment decisions are from genomic origin.

Immunotherapy in breast cancer

TNBC, characterized by the absence of estrogen receptor, progesterone receptor, and HER2 expression, poses a formidable challenge in breast cancer treatment due to limited targeted therapeutic options. Immunotherapy has emerged as a promising strategy to address this unmet clinical need (53). Indeed, advancements in immunobiology have paved the path for effectively boosting host immunity in the fight against breast cancer. By leveraging the host immune system, immunotherapy aims to activate and enhance the antitumor immune response (53, 54). Immune checkpoint inhibitors, such as anti-PD-1/PD-L1 agents, have shown potential in unleashing T cell-mediated immune responses against TNBC cells, and increased antitumoral immune response in preclinical studies (54). Additionally, adoptive T cell therapies and therapeutic cancer vaccines are being explored to augment the antitumor immune response. While certain TNBC cases have exhibited remarkable responses to immunotherapy, challenges such as inherent or acquired resistance, tumor heterogeneity, and identifying predictive biomarkers necessitate further investigation (53, 54). To this goal, combining immunotherapy and conventional treatments could be a new way forward to enhance treatment efficacy.

Metabolomics of breast cancer

Metabolomics is the measurement of the aggregate metabolic outcome of biological systems, which establishes a reflection of dynamic cellular functions due to pathophysiological stimuli and genetic variations (55, 56). A metabolome is a quantitative collection of small molecules, called metabolites, present in a biological sample (blood, urine, saliva, serum) at a specific time. It is the representative of all cellular processes, and encompasses a wide range of compounds such as lipids, amino acids, sugars, nucleotides, and various other metabolites involved in cellular processes (57).

Metabolomics, viewed as a consequential stage of proteomics, transcriptomics, and genomics, offers promise as a potentially non-invasive liquid biopsy approach. In the future, it could be employed for cancer diagnosis and characterization, for monitoring treatment response and toxicity, as well as for predicting outcomes from the initial stages of diagnosis (55).

Metabolic fingerprint of a metabolome

A possible explanation of the connection between metabolome and genome (with the first being dependent on the latter) is as follows: one's genetic information is contained in the genome as DNA sequences, which are transcribed into RNA (transcriptome), which in turn is translated into a protein (proteome) that undergoes metabolism, ultimately forming small molecules, called metabolites, in a metabolome. Therefore, any genetic alteration—such as mutation, over-expression, deletion or insertion—may cause significant changes in the metabolic profile. These genetic changes that alter the metabolic profile also step up cancer development (56).

The metabolomic constitution is also influenced by various other factors, both from internal and external sources, including age, gender, race, diet, physical activity, health state, and drug exposure (58). Consequently, the distinct patterns of individually expressed metabolites form a unique metabolic fingerprint, indicating the idiosyncratic biological configuration of that individual's metabolic process (55).

In a wide-ranging study on metabolomics, operating 928 cell lines from more than 20 diverse cancer types, researchers discovered 225 metabolites that are distinctive to cancer metabolism. Similarly, substantial evidence links obesity and physical inactivity to an elevated risk of developing breast cancer (58). As alterations in metabolomic profiles can be linked to various pathological conditions, the study of the metabolomic biomarkers will play a crucial role in advancing personalized medicine as well as enabling early detection and biological characterization of diseases, especially cancers (55, 56).

The analysis of a metabolome is performed by three main techniques. These are nuclear magnetic resonance (NMR) spectroscopy, gas chromatography-mass spectrometry (GC-MS), and liquid chromatography mass spectrometry (LC-MS) (56, 57). Moreover, scanning electron microscopy (SEM), matrix-assisted laser desorption ionization (MALDI), and nanostructure-imaging mass spectrometry (NIMS) have also been employed for metabolome investigation (56).

Metabolomic profile in breast cancer

Tumor cells exhibit notable deviations in cellular processes and altered metabolites compared to normal cells. Notably, altered metabolic pathways in malignant cells are widely acknowledged. One considerable example is the use of aerobic glycolysis instead of the typical mitochondrial oxidative phosphorylation to produce adenosine

triphosphate (ATP), a well-known phenomenon referred to as the "Warburg effect". This adaptation of cancer cells is believed to provide an advantage to survive even in hypoxic conditions (57).

It is evident that cancer cells require ample supply of nutrients for sustenance and growth. They use this energy for proliferation, angiogenesis and epithelial-to-mesenchymal transition (EMT) (56). Distinct metabolic profiles in different subtypes of BC also aid in their classification: for example, comprehensive analysis of glutamine to glutamate ratio in tumor tissue has revealed links with estrogen receptor status, tumor grade, and overall survival (57). Similarly, metabolites from other energy-generating pathways are found in higher levels in triple-negative BC than in hormone-receptor positive BCs, and it suggests aggressiveness (56).

Irrefutably, metabolomic analysis led to the discovery of potential biomarkers for early diagnosis and tailored therapy that warrant further validation.

Mitochondrial dysfunction in BC

Mitochondria possess their own genome, called mitochondrial genome (mtDNA, or mitogenome), whose transcription and translation are controlled by various mechanisms. It has been evidenced that there is an efficient epigenetic system—containing methylated DNA/RNA bases, a network of noncoding RNAs, and posttranslational mechanisms of histone modification—that controls gene expression of mtDNA. An established cross-talk mechanism has been observed between nuclear and mitochondrial genomes, which is also responsible to control the mitochondrial activity (59).

mtDNA is very vulnerable to damage by nuclear DNA mutations, leading to the malfunctioning of the mitochondria-operated respiratory chain and energy production and consequently promoting the generation of additional reactive oxygen species (ROS), which sponsors oncogenicity. Reliable studies evidenced that aberrations in mitochondrial genome are also involved in breast cancer origination and progression (56). Unstable mitochondrial epigenetics (mito-epigenetics) and defective governance of oxidative phosphorylation processes potentially foster the growth of cancer cells. Therefore, these alterations and mutations in mtDNA can be a powerful target for anti-breast cancer therapies. Different mitochondria-centered treatments have been tested in breast cancer clinical trials. These include OXPHOS inhibitors, antibiotic bedaquiline, biguanides, vitamin E analogs, etc. (59).

Lipid metabolism in BC

Cancer cells essentially rely on ample lipid supply and metabolism for their growth and proliferation: besides providing energy, lipids form the structural foundation of cell membranes, serve as energy reservoirs, and also act as signaling molecules. Cancer cells use fatty acids, which are like building blocks made of lipids, in two possible ways: either for lipogenesis (i.e., to synthesize many lipid molecules for tumor growth), or to transport many fatty acids into the cells to generate energy, to be used by cancer cells by beta oxidation. In numerous cases, the presence of metabolites responsible to cause fatty acid metabolism and transport is indicative of breast cancer, and they can serve as biomarkers for early detection (56, 59).

Another lipid metabolic alteration is the presence of higher acylcarnitine C2 levels, which is associated with increased risk of breast cancer. Acylcarnitine C2 facilitates the transfer of fatty acids into the mitochondria and its abundance is an indicator of excessive lipid availability and heightened fatty acid oxidation in breast cancer (58).

There is an association between cancer cells and adipocytes that favors oncogenesis. Adipocytes secrete a hormone, called leptin, which—like insulin—was found to facilitate breast cancer growth, thus making hyperleptinemia an important metabolic indicator in the pathophysiology of breast cancer (61). Similarly, high levels of CD36, a fatty acid transporter that facilitates the influx of exogenous fatty acids, exhibit increased protein expression in various cancer types, including BC (60). In light of these findings, it is apparent that BC cells show eminent dependency on fatty acid and lipid metabolism to grow and multiply. Hence, the aforementioned metabolic pathways can be pharmacologically inhibited to mitigate breast cancer.

Carbohydrate metabolism in BC

Cancer cells use carbohydrates as their primary energy source, but they prefer aerobic glycolysis for glucose metabolism, even when the oxygen exists (Warburg effect). Increased glucose uptake by cancer cells is due to the overexpression of oncogenes RAS and MYC, and mutation in tumor suppressor gene TP53, which leads to high proliferation and decline of apoptosis (62). Elevation in various processes like glycolysis, glycogenolysis, redox pathways, and TCA cycle is not just for increased energy production, but they also release certain metabolites that act as precursors for many macromolecules (56): for example, glucose-6-phosphate metabolizes to give precursor molecule ribose-5 -phosphate for nucleic acid biosynthesis, and intermediate 3-phosphoglycerate is a precursor for amino acids glycine and cysteine. So, excess generation of precursor molecules promotes cancer biomass and proliferation (56).

Glucose transporter proteins (GLUT1-5) are highly expressed in breast cancer; specifically, GLUT-1 is excessively expressed in TNBC patients (63). Nowadays, several GLUT-1 inhibitors (like BAY-876) have been implemented as potential targeted therapeutics to specifically inhibit TNBC cell lines (64). Additionally, excessive release of lactate in the tumor microenvironment, as a result of Warburg effect, makes the microenvironment acidic, which eventually encourages tumor progression, angiogenesis, metastasis, and essentially immunosuppression, thus leading to adverse outcomes (65, 66). Clinical investigators found out lactate to be an oncometabolite, as it amplifies the expression of genes involved in cell division, cell proliferation, and elevated transcription in human breast cancers (66).

Briefly, carbohydrate metabolites play a major role in amplifying breast cancer and demand researchers' attention to develop targeted therapies (56).

Amino acid metabolism in BC

Like any other cancer, breast cancer also uses amino acids for cell proliferation and persistence. Collectively,

essential, semi-essential, and non-essential amino acids play key functional roles inside the cells, such as epigenetic modifications, α-ketoglutarate production (which acts as a substrate for TCA cycle), ATP production, protein synthesis, glucose and lipid metabolism, and signaling pathways (56).

In this regard, glutamine and its intermediate metabolites (NADH and glutathione) are vital for cancer growth, as they meet energy demands and help in combating oxidative stress in tumors. Sometimes cancer cells undergo glutamine addiction, because they are unable to survive when glutamine is lacking (63). Therefore, cancer cells exhibit increased expression of glutamine transporters ASCT2, SNAT1, SNAT2, and SNAT5 for its sufficient influx. This crowded glutamine enters the cell, first gets converted into glutamate, and then undergoes metabolism through TCA cycle to generate an immense amount of energy for cancer cells by the process of glutaminolysis (56). This process also releases some macromolecules that cancer cells use when lacking glucose—like citrate, malate, and fumarate. Reduced glutamine metabolism encourages lipid biosynthesis, which in turn favors tumor cells in hypoxia or mitochondrial dysfunction. Therefore, glutamine promotes carcinogenesis, even when nutrients are inadequate in the microenvironment. The key enzyme glutaminase, converting glutamine into glutamate, is a potential target for breast cancer treatment (67). Its inhibitors have given successful results in diminishing tumor growth in TNBC cell lines. In addition, the inhibition of ASCT2 (a glutamine transporter) also turned out to be successful in halting tumor growth in TNBC patients (56).

Similarly, serine transporter ASCT1 is also highly expressed in breast cancers. Cancer cells highly depend on the availability of extracellular serine, which they use for nucleotide synthesis and DNA methylation. Moreover, an overexpression of serine biosynthesis genes is also associated with breast cancer metastasis to bones and stimulates osteoclastogenesis (68). Minimization of serine levels can prevent the growth of cancer cells.

Likewise, homocysteine, cysteine, and branched chain amino acids like leucine, iso-leucine, and valine are also crucial for cancer cell proliferation. Especially, a cysteine excess can cause oxidative damage and produce free radicals, which become sources of gene mutations. Additionally, higher plasma cysteine levels indicate the risk of breast cancer and helps in early diagnosis (69). Tryptophan and L-arginine disturbs immune regulation and potentially promotes the growth of breast cancer cells, and thus their suppressants contribute to halt the tumor growth (63). Targeting amino acids metabolism could thus be useful in preventing and treating breast cancers.

One of the hallmarks of cancers is metabolic reprogramming, which allows researchers to predict whether the initially observed premalignant lesions may proliferate or metastasize in future. This creates opportunities to pinpoint the metabolic dependencies of BC, in order to overcome its pathogenesis and overall burden.

Differential metabolomic fingerprints for breast cancer subtypes

Alterations in the gene expression profile cause changes in the metabolic profiles of BC subtypes. For example, luminal subtypes emerge by alterations in GATA3 and PIK3CA mutations, whereas TP53 mutations give rise to basal-like BC (12). Therefore, each BC subtype exhibits variations

Table 2. Metabolic signatures in various molecular subclasses of breast cancer

	Luminal A	Luminal B	HER-2	Basal-like (TNBC)	Reference
IHC status	(ER+) (PR+) (HER2-)	(ER+)(PR+)(HER2+)	(ER-)(PR-)(HER2+)	(ER-)(PR-)(HER2-)	
Glucose metabolism	High glucose consumption, high lactose production, poor TCA activity, slow glycolysis, locally multiplied cells	Low glucose consumption, high lactate production, efficient glycolysis, aggressive cancer	Increased glucose consumption, high lactate production, enhanced glycolysis, low oxygen consumption, more aggressive than luminal subtypes	Higher glycolysis, high lactate accumulation, lower oxidative phosphorylation than luminal subtypes, highly invasive breast cancers	(70)
Amino acid metabolism	Low expression of GDS and GLD, decreased glutamate-to-glutamine ratio	High expression of GDS and GLD, decreased glutamate-to-glutamine ratio	High expression of GDS and GLD, higher glutamate-to-glutamine ratio, active glutaminolysis	Higher glutamate-to-glutamine ratio, active glutaminolysis	(71)
Lipid metabolism	Upregulation of de novo FA synthesis, mobilization, and oxidation	Upregulation of de novo FA synthesis, mobilization, and oxidation	Highest expression of lipid metabolic proteins, increased de novo FA synthesis	Expression of lipid metabolism protiens is slightly lower than other subtypes, increased de novo FA synthesis	(72, 73)

Abbreviations: ER, estrogen receptor; PR progesterone receptor; HER2, human epidermal receptor2; TNBC, triple-negative breast cancer; TCA, tricarboxylic acid; GDS, glutamate dehydrogenase; GLD, glutamate decarboxylase; FA, fatty acid.

in metabolic alterations or metabolic signatures that can be helpful in predicting the possible therapeutic strategy. Moreover, metabolomic analysis and subtype differentiation can be simply performed through plasma samples, which sidesteps the hustle of performing a biopsy.

In this context, the distinct metabolic fingerprints of each subtype are now presented in **Table 2**. This compilation is made by extensive review of studies and could serve as a comprehensive reflection of the metabolic profile of each subtype.

Microbiomics of breast cancer

Human bacterial composition causes noticeable alterations in the normal functions of the body, such as metabolic changes, inflammation, allergy, and cancer progression. In the recent years, much attention has been given to the characterization of the microbiota from different parts of the body—including gut, skin, urinary tract, and other organs—to correlate their potential in stimulating carcinogenesis. Each organ exhibits a distinct microbiota, which leads to well-defined pathological findings, including cancer (74). Any alteration in the microbiome ecology of each specific organ leads to **"dysbiosis"**, a phenomenon that causes disease, pathological conditions, and tumorigenesis (75).

Currently, research on breast microbiota has discovered a unique bacterial population, which is important for breast health because these bacteria may possess either pro- or anti-carcinogenic properties. Next generation sequencing (NGS) techniques are a powerful tool in revealing bacterial signatures in breast health and their role in carcinogenesis. Additionally, they may serve as important biomarkers and targets for BC therapies (76).

Mechanisms by which dysbiosis induces breast cancer

Several pathways have been discussed in the literature, through which breast tissue microbiomes can induce tumorigenesis.

The first possible way is the incitement of chronic, dysregulated inflammation, which can lead to malignancy. Dysbiosis destroys the host's immune regulation and leads to tumor-promoting inflammation. Studies have shown that cancer patients show a decreased lymphocyte count, associated with disease relapse and high mortality (73, 75).

Secondly, the diverse microbial population in human gastrointestinal (GI) tracts is responsible for influencing estrogen metabolism. Endogenous and circulating estrogen is the key hormone accredited to cause breast cancer. After performing its assigned roles in sexual cycle regulation, estrogen is inactivated by the liver and excreted through the intestines. However, certain bacteria present in the gut are able to deconjugate the hormone by the activity of enzymes β-glucoronidase and β-glucosidase, and free estrogen is absorbed again into the blood. Its abundance in circulation is linked with increased risk of breast cancer, especially in postmenopausal women (74, 75, 77). Researchers coined the term 'estrobolome' for all enteric bacterial genes that influence oestrogen metabolism. This estrobolome can be helpful in prognosis of ER-positive BC, and thus can be targeted via appropriate antibiotics (75, 77).

Moreover, an unhealthy diet with high fat and low fiber, along with dysbiosis in the gut microbiota, also leads to obesity, which is a potential risk factor for BC. Studies have shown that certain bacteria, like *Firmicutes* and *Bacteroidetes*, bring about metabolism of dietary fibers and polyphenols. Their decline in the GI-tract could lead to obesity and increased estrogen levels (75).

Next, some microbes are able to cause genomic instability and double-stranded breaks in DNA by producing colibactin, a genotoxin produced by certain *E. Coli* strains. This indicates complex interactions between gut bacteria and breast cancer (78).

Few studies also examined the difference between the microbiomes of BC patients and healthy controls. One study revealed that non-cancerous women harbor *Methylobacterium* in larger amounts in breast tissues than their BC counterparts. Moreover, urine sample comparison between the groups revealed differences in microbiomic profiles: cancer patients were found to carry more abundant gram-positive bacteria in their urinary tract than the control group (79, 80).

However, even though numerous studies demonstrate correlations of bacteria with BC, their exact causative roles are still unclear, and the precise mechanisms powered by such bacteria are still to be fully understood.

Microbiome and bacterial therapy for breast cancer

Classical treatments to exterminate breast cancer include surgery, radiotherapy, chemotherapy, and some modern approaches like immunotherapy, stem cell therapy, dendritic cell-based therapy, hormonal therapy, and so on. However, all of these methods have their own limitations in clinical practice. For instance, chemotherapy induces nonspecific toxicity towards the host's normal cells, and also their multidrug resistance. This is why genetic engineering has yielded genetically modified non-pathogenic bacterial strains that are selective for cancer cells, with lower toxicity and fewer side effects. Bacteria-mediated tumor therapy (BMTT) is a potential therapeutic approach for breast cancer, on the way of its refinement, which is gaining the attention of researchers and giving promising results (81, 82).

Previously, the role of carcinogenic bacteria has been extensively reported in the literature, highlighting their capability to enhance tumor progression. However, recently, some bacterial species have demonstrated great potential to invade and colonize solid tumors, resulting in growth retardation and, occasionally, even complete eradication of cancer. Examples of such strains include *Clostridia*, *Bifidobacteria*, *Shigella*, *Vibrio*, *Escherichia*, and *Salmonella* (81, 82).

Bacteria can hinder with tumor progression in many ways, some of which are listed below:

Bacteria like *E. Coli* are used for host immune response stimulation. Stimulated T-lymphocytes are associated with antitumor activity (81);

Bacterial products like toxins, bacteriocins, and enzymes produced by specific bacterial strains have shown oncolytic properties. For example, bacteriocin Bovicin HC5 demonstrated anti-cancer activity in breast cancer cell lines in vitro.

Additionally, Laterosporulin 10 (LS10) is a peptide produced by gram-positive bacteria that can induce necrosis and cell death in breast cancer cell line MCF-7 (81);

Sometimes attenuated strains of bacteria (e.g., *Salmonella*) have also been used for cancer bacteriotherapy, in which bacteria are used as vehicles to target human tumors. For example, attenuated bacteria of *Salmonella typhimurium* produce antitumor activity against different cell lines of breast cancer (81).

Conclusions

Interest in the multi-omics approach to study breast cancer has increased in recent years, as it has become discernable that the relationship of genome, proteome, metabolome, and microbiome in BC can reveal novel biomarkers and therapeutic targets. Moreover, omics studies led to the discovery of specific genomic and metabolomic features of BC that are helpful in categorizing BC patients and identifying disease progression and response to treatment. For instance, the unique metabolic profile of BC patients is highly sensitive to the microbiota of breast tissue microenvironment. Therefore, analysis of metabolic changes in BC with respect to micro-biomic implications can provide new insights into treatment modalities. In conclusion, multi-omics has emerged as an innovative, promising approach for profiling specific omics features associated with BC, and demands solicitous large-scale future research.

Acknowledgments

This research was funded by the Provincia Autonoma di Bolzano in the framework of LP 14/2006.

Conflict of Interest

Authors declare no conflict of interest.

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