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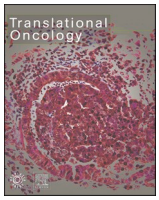

Non-genetic heterogeneity and immune subtyping in breast cancer: Implications for immunotherapy and targeted therapeutics

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ABSTRACT

Breast cancer (BC) is a complex and multifactorial disease, driven by genetic alterations that promote tumor growth and progression. However, recent research has highlighted the importance of non-genetic factors in shaping cancer evolution and influencing therapeutic outcomes. Non-genetic heterogeneity refers to diverse subpopulations of cancer cells within breast tumors, exhibiting distinct phenotypic and functional properties. These subpopulations can arise through various mechanisms, including clonal evolution, genetic changes, epigenetic changes, and reversible phenotypic transitions. Although genetic and epigenetic changes are important points of the pathology of breast cancer yet, the immune system also plays a crucial role in its progression. In clinical management, histologic and molecular classification of BC are used. Immunological subtyping of BC has gained attention in recent years as compared to traditional techniques. Intratumoral heterogeneity revealed by immunological microenvironment (IME) has opened novel opportunities for immunotherapy research. This systematic review is focused on non-genetic variability to identify and interlink immunological subgroups in breast cancer. This review provides a deep understanding of adaptive methods adopted by tumor cells to withstand changes in the tumor microenvironment and selective pressure imposed by medications. These adaptive methods include alterations in drug targets, immune system evasion, activation of survival pathways, and alterations in metabolism. Understanding non-genetic heterogeneity is essential for the development of targeted therapies.

Introduction

Breast cancer (BC) is the most common cancer among females globally and has several subtypes. Among females, it accounts for 30 % of all cancers with a 15 % mortality rate worldwide [1]. The prevalence of breast cancer varies worldwide, as 27 cases per 100,000 humans are affected in East Asia and Africa but 97 cases per 100,000 individuals in North America [2]. These geographical differences in the prevalence of cancer indicate the relationship between BC, the growth of the economy, and distinct social and lifestyles. Every woman should have proper access to early diagnostic, high-quality preventative and treatment services to reduce BC burden [3]. BC has extraordinary variability. Clinically, it is classified as 1) estrogen receptor (ER), 2) progesterone receptor (PR), and 3) HER2 based on particular proteins that are identified by immunohistochemistry expression. Based on immunohistochemistry expression, BC is divided into four major categories: HER2-positive, PR-positive ER-positive, and triple-negative breast cancer (TNBC), which lacks all these three receptors [4].

The uncontrolled division of cancerous cells is affected by hereditary and non-genetic factors and is the major hurdle in cancer diagnosis and therapy. Treatment of BC can be complicated due to variations in phenotypes and genotypes of patients. Variation in phenotypes of cancerous cells is regulated by the signals from dynamic microenvironment, differences in components of cells, and cellular state. Many factors are responsible for this variability including non-genetic factors [5]. Non-genetic variation factors that are responsible for functional and phenotypic variability include transcriptional pulsing or bursting and stochastic distribution of the components in cell division [6]. It is

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hypothesized that these biological incidences represent internal variations or modifications in cell state that sustain non-genetic heterogeneity (Fig. 1).

Recent advances in systemic treatment of breast cancer have significantly changed treatment protocols, resulting in improved clinical outcomes. A breakthrough was the introduction of targeted therapies that focus on specific molecular abnormalities in cancer cells. For example, new agents targeting the HER2 and CDK4/6 pathways have demonstrated significant efficacy in clinical trials, resulting in improved progression-free survival and life quality of breast cancer patients [7]. Additionally, the introduction of immune checkpoint inhibitors has opened new ways for advanced breast cancer therapy, providing satisfactory responses in a group of patients who previously had limited treatment options [8]. The move toward personalized medicine is another important trend in the emerging breast cancer treatment landscape. Molecular profiling techniques, such as next-generation sequencing, can identify genetic variants and mutations that may be amenable to specific therapies [9]. This approach not only works on the treatment of each patient according to his tumor biology but also ameliorates its effectiveness and minimizes associated toxicity associated with traditional chemotherapy. Moreover, recent studies have highlighted the benefits of combining new agents with already existing therapies to overcome resistance and improve disease control. Binding PARP inhibitors with immune checkpoint inhibitors significantly increased anti-tumor effects and improved survival rates in BRCA-mutated BC patients [10]. These developments in drug discovery have shown an innovative approach to the treatment of BC with more effective and target-specific treatment options.

Studies on cancer have changed significantly. Although treatment resistance and tumor evolution were traditionally thought to be primarily caused by genetic changes, new findings have painted a more complex picture. Transcriptional reprogramming and epigenetic changes are considered the primary components of this mechanism. Cancer cells can survive in response to the therapies because these mechanisms can alter gene expression patterns without altering the underlying DNA sequence. Researchers are acquiring information about the molecular basis of cancer progression by deciphering the complexities of epigenetic regulation and transcriptional dynamics. This information facilitates the development of cancer treatment by targeting these mechanisms [11,12]. This review is focused on the investigation of the role of non-genetic factors in intratumoral heterogeneity and treatment resistance in breast cancer. We aim to unravel the resistance mechanism that goes beyond genetic modifications by investigating many molecular and cellular features in cancer. This study aims to formulate targeted therapies and personalized treatment methods that significantly combat intratumoral heterogeneity, eventually enhance patient outcomes, and overcome treatment resistance.

Non-genetic heterogeneity in breast cancer

The identification of distinct phenotypic characteristics within BC cell subpopulations highlights a critical aspect of non-genetic cellular heterogeneity [13]. Numerous tumor subtypes, immune cells, and stromal cells can cause breast cancer; each has unique phenotypic, genetic, and epigenetic traits, creating a mosaic-like structure. Recent studies exploring structural modifications, changes in epigenomic patterns, and disruptions in transcriptomic regulation, alongside the immune context, have highlighted the substantial functional heterogeneity within tumors, resulting in notable impacts on their behavior and characteristics. The presence of heterogeneous tumor cell subpopulations facilitates the process of selection and Darwinian evolution, allowing for the survival and proliferation of advantageous traits. Moreover, this heterogeneity enables beneficial cooperative interactions among tumor cells, which can lead to the advancement of tumor progression and potentially lead to resistance against therapeutic intervention [14–16]. Genetic variation serves as a primary inducer of cancer development through clonal evolution [17]. Moreover, the capacity of cancer cells to acquire different phenotypes without changing their genome represents non-genetic adaptation in response to intrinsic or extrinsic factors. This process ultimately results in the development of a heterogeneous phenotype within the tumor [18].

Heterogeneity is not solely confined to genetic alterations like somatic cell changes and chromosomal abnormalities. Non-genetic factors also contribute significantly to cell-to-cell phenotypic variability within tumors. These non-genetic sources of heterogeneity encompass variations in epigenetic patterns, transcriptomic profiles, proteomic compositions, and metabolic characteristics among tumor cells, even if they acquired similar genetic variations [19]. Epigenetic variations, including hypermethylation of promoter regions, modified enhancer activity, and changes in the configuration of chromatin, collectively play a crucial role in cancer development and have a broad impact on gene expression. Changes to the cancer epigenome can exhibit two distinct patterns: binary alterations that act as either "on" or "off" switches for gene expression, and transient changes that lead to fluctuations in gene expression as part of dynamic and flexible gene expression networks. Furthermore, epigenetic modifications may affect copy number changes, leading to the complexities of cancer development and progression [20, 21].

The cellular transcriptome, like the epigenome, frequently reveals modifications in cancer. Modified splicing changed promoter applications, gene fusions, and aberrant oncogenic signaling are all possible causes of these abnormalities. These mechanisms can cause major alterations in gene expression patterns, resulting in deregulation of biological functioning in cancer cells [22–26].

Cancer cell heterogeneity caused by cellular plasticity accounts for a

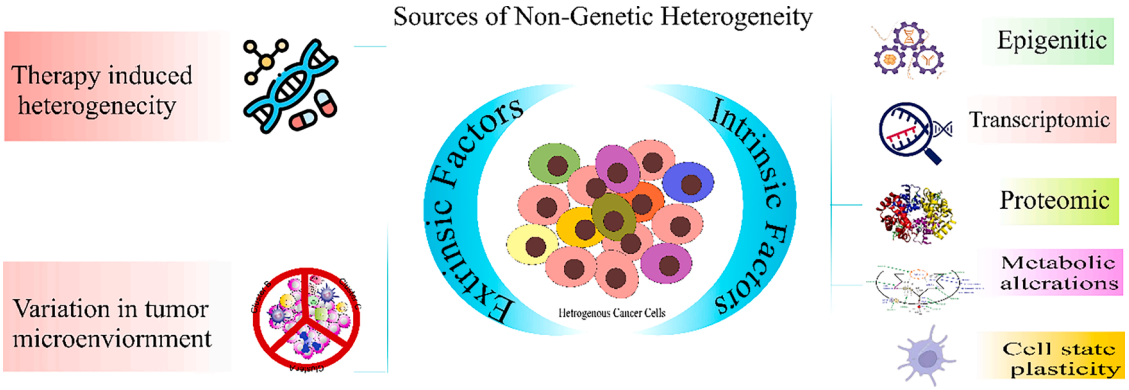

Fig. 1. Sources of non-genetic heterogeneity. Intrinsic sources of non-genetic heterogeneity are 1) epigenetics, 2) transcriptomic, 3) proteomic, 4) metabolic alterations, and 5) cell plasticity. While extrinsic variables have variations in signaling pathways which are caused by changes in the tumor microenvironment or therapy-induced changes.

considerable diversity in breast cancer. An excellent example is the epithelial to mesenchymal transition (EMT). Rather than possessing distinct populations of epithelial or mesenchymal carcinoma cells, BCs have a variety of cells that exhibit both epithelial and mesenchymal properties [26]. The ability of a cell to shift identity results in intra-tumor heterogeneity. Intra-tumor heterogeneity is influenced by population noise, temporal heterogeneity, and cell-to-cell variation within a population, which describes changes in a property over time in an individual cell. Both types of heterogeneity play concurrent roles in determining cell fate and function, and these complexities have significant implications for cancer therapy [27]. Tumor cell clonal heterogeneity and cooperative plasticity confer considerable functional adaptability to tumors, potentially augmenting tumor growth and facilitating metastasis.

In addition to changes in cell state, the tumor microenvironment is a major factor in promoting heterogeneity, particularly of non-genetic origin [28]. The survival and propagation of specific subpopulations of cancer cells, as well as the transitions between these subpopulations, are influenced, at least in part, by the tumor microenvironment. The microenvironment plays a crucial role in shaping the behavior and characteristics of cancer cells [29]. The selection of certain subpopulations of cancer cells that are better adapted to specific microenvironmental conditions is referred to as Darwinian selection. This process is responsible for driving non-genetic heterogeneity within the tumor. Solid tumors, like breast cancer, often exhibit a heterogeneous microenvironment. This heterogeneity in the microenvironment further contributes to the overall burden of heterogeneity within the tumor [18] (Fig. 1).

In the present era, precision or personalized medicine is strongly advocated to address the functional and structural diversity observed among individual patients. To overcome intratumoral heterogeneity, a significant challenge in BC, researchers are increasingly leveraging multi-omic profiling, encompassing proteomic, genomic, and metabolomic data, particularly at the single-cell level. This comprehensive approach has the potential to refine both prognostic and therapeutic strategies for BC management [30]. Furthermore, a systematic investigation of the molecular underpinnings of metastatic BC heterogeneity, a major factor contributing to therapeutic resistance, could result in the emergence of more efficacious antimetastatic agents and improve patient outcomes [31].

The interaction between the tumor microenvironment and cancer cells is extremely complicated, and it has a considerable impact on the heterogeneity and behavior of cancer populations. Understanding these interactions is critically important for the development of targeted drugs.

Immune system's role in breast cancer progression

BC originates in the tissue microenvironment (TME) which possesses many stromal cell types embedded in the extracellular matrix. ECM is responsible for structural support and cellular communication in the tumor site. Immune cell infiltration significantly changes in breast epithelium with the progression of cancerous cells from normal cells. Immune cell populations are altered quantitatively and qualitatively in both the epithelial and stromal compartments during the carcinogenic process [32].

Immune infiltrate in BC is the heterogeneous population that consists of a variety of immune cell subtypes such as T lymphocytes (further classified as CD3+ with subpopulations CD4+ and CD8+), monocytes/macrophages, B lymphocytes, natural killer (NK) cells, and dendritic cells [33]. The chemical composition of immune infiltrate in the tumor microenvironment significantly affects BC progression and treatment response. This intratumoral heterogeneity which is characterized by the co-localization of multiple immune cell subtypes in epithelial and stromal compartments, provides intricate interactions between tumor cells, immune cells, and other TME components [34]. Consequently, they can significantly impact tumor growth through various mechanisms. This impact either may be directly through cytotoxicity mediated by CD4+ and CD8+ cells or indirectly via immunostimulatory or immunosuppressive consequences caused by the secretion of growth factors, cytokines, and other agents [35]. The intricate relationship between immune cells and tumor microenvironment plays a major role in defining the behavior of tumors and responsiveness to therapies.

The immunogenicity of BC is different among its molecular subtypes, as it is the highest in TNBC and HER2-positive tumors, while its level is lower in luminal A and luminal B subtypes [36]. Moreover, the greater number of tumor-infiltrating lymphocytes (TILs) is correlated with a better response to neo-adjuvant therapy and an improved prognosis in breast cancer patients. This finding demonstrates that a high intratumorally immune response can significantly play a role in cancer progression [35]. Tumor-infiltrating lymphocytes have prognostic significance [37]. TIL collection and infusion have also led to long-term full regression of compact tumors, and advancements in cellular therapy by using gene-modified T cell receptors for the treatment of breast cancer and other tumors. TILs play a major role in the immune response to tumors and have tremendous potential for the development of new cancer therapies [37,38].

Although PD-L1 expression may be closely linked to response to checkpoint inhibition, there are many hurdles to using it as a diagnostic biomarker. This was observed in the KEYNOTE-86 pembrolizumab trial in TNBC patients, where PD-L1 status did not appear to be the best indicator of responders and non-responders [39]. This demonstrates that many other factors may influence the response to checkpoint inhibitors in TNBC and other biomarkers or combination methods may be essential to increase the prediction accuracy.

The immune checkpoint inhibitors have resulted in a significant paradigm shift in immune-oncology treatments in recent years. Targeting PD-1 and PD-L1 by blocking antibodies has demonstrated significant potential for controlling cancer [40]. These results showed the role of immune cells in breast carcinogenesis. In addition, these immune cells undergo various changes with the development of BC and highlight the dynamic role in the onset of diseases. Based upon this context, immunotherapy has emerged as a promising strategy in the treatment of BC using the relationship between the immune system and tumor microenvironment to improve patient outcomes and to develop novel therapeutic strategies.

Immune subtyping of breast cancer

BC is classified on the basis of histology and immunohistochemical analysis. Histological aspects disclose the cellular and tissue structure of the tumor, while immunohistochemistry studies demonstrate the presence or absence of specific protein markers including progesterone receptor (PR), estrogen receptor (ER), and HER2. Moreover, BC can be categorized by using gene expression analysis based on intrinsic gene expression patterns [41–43]. Clinicopathological factors such as grade, molecular subtypes, stage, and age played a major role in prognosis and therapy. Approximately 90 % of all BCs are divided into two subtypes; invasive lobular carcinoma (ILC) and invasive ductal carcinoma not otherwise defined (IDC NOS) according to the histological findings [44]. Molecular profiling has emerged as an intriguing approach to analyzing tumors, providing insights into responses to many therapies. Five molecular subtypes of BC having significant clinical implications are revealed by advanced gene expression. These subtypes are luminal A, luminal B, HER2-enriched, normal, and basal, and possess unique properties in terms of survival rates, incidence, underlying tumor biology, and prognosis [45,46]. This patient classification based on the molecular subtypes has significant clinical and economic benefits in the treatment of breast cancer [47]. Molecular variations, either intra-tumor or inter-tumor, prevent the implementation of the standard therapy [48]. Systematic therapy varies among the patients based on their specific subtypes.

Furthermore, a complex interaction between the tumor cells and the surrounding microenvironment significantly affects the cancer progression, development, and therapeutic responses. Different types of cells and signaling molecules are present in this microenvironment which are responsible for genetic and epigenetic variations in tumor cells, eventually affecting cancer incidence and then treatment resistance [49]. Cancer progression including BC is affected by this tumor microenvironment [50,51]. There is a complex ecology of chemicals and cells in the tumor microenvironment which are critically important for immune response. Immune cells of TME can kill tumor cells or enhance tumor advancement by recognizing them [52]. It is very important to identify the structure and functions of tumor immune infiltration for various reasons. Firstly, it identifies which patients will benefit from immunotherapy. Secondly, analysis of the complicated relationship between the tumor and host immune responses clarifies the progression of cancer and the development of treatment methods [53]. The understanding of the tumor microenvironment and its role in cancer variability is significantly important to develop customized and targeted therapies for BC.

Various subgroups of breast cancer have been identified. These subtypes are categorized on the basis of nature and number of immune cell infiltrates as illustrated in Fig. 2. Results demonstrated that immunological clusters have independent predictive value in breast cancer which emphasizes the interaction of the tumor phenotype and immune contexture [54]. Moreover, the hierarchical clustering analysis by using myeloid and lymphoid immune cell populations showed the differences in their immunological context in breast cancer intrinsic molecular subgroups. This study suggests that combining the immune contexture with already established prognostic markers like PAM50 and clinical tumor load can enhance the patient risk classification for breast cancer and potentially improve prognostic models [55].

Tumor tissue microenvironment (TME) affects the immunotherapeutic therapy response. In general, cancer cells characterized by high T cell infiltration, known as "hot" tumors, tend to respond better to immunotherapies compared to "cold" tumors with low T cell penetration [56]. A recent study utilized a novel immunological classification system for BC based on both bulk and single-cell transcriptome data. This approach identified three distinct breast cancer-BC subtypes: BC-ImH, BC-ImM, and BC-ImL. These subtypes were distinguished based on their immunological signature scores, with BC-ImH showing the highest score, indicating a strong immune response, and BC-ImL displaying the lowest score, indicating a suppressed immune microenvironment [57]. In this study, the BC-ImH subtype correlated with PD-L1 expression, tumor mutation burden, and high quantity of TILs, all of which are biomarkers for a positive ICI response. For example, HER2+ BC and triple-negative breast cancer are more immunogenic, but HR + BC is less immunogenic. Therefore, immunotherapy may be a feasible choice for patients with HER2+ or HR+ BC and TNBC. Many studies have shown the role of immune subtypes in predicting the response to treatment of TNBC and other cancers with immunosuppressive carcinomas [58,59]. Additionally, major myeloid cell populations have identified unique immunological subgroups of triple-negative breast cancer-TNBC [60]. Two subtypes were reported, the neutrophil-enriched subtype and macrophage-enriched subtype. More unique was the development of descriptive dichotomous myeloid compartments within each subtype marked by profound differences in frequency, function, and therapeutic potential of these cells. While macrophage-enriched subtype (MES) tumors displayed various responses to immune checkpoint blockade (ICB), some remained responsive, while others demonstrated no response, neutrophil-enriched subtype (NES) tumors, by contrast, were uniformly resistant. Furthermore, the research demonstrated that initially, ICB-sensitive MES models could develop resistance through a transition to the NES phenotype, highlighting the potential clinical significance of these subtypes [60,61].

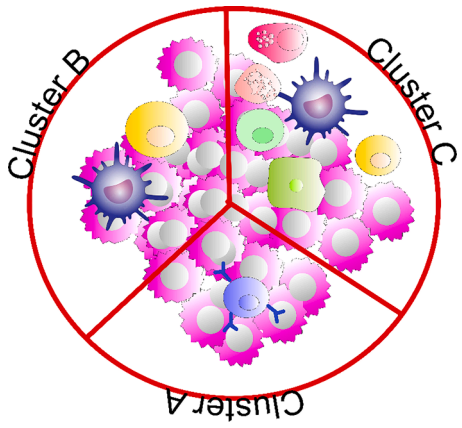

Fig. 2. Immune-related subtypes of breast cancer. Cluster A is classified as the Immune Cold and shows low immune infiltration, Cluster B is labeled as Protumorigenic (Promoting tumorigenesis) while Cluster C is termed as Immune Hot and exhibits a high presence of the activated immune cells.

Gaining more insight into the intricate relationship between immunological microenvironment and breast cancer is crucial. These hold promise for the development of targeted immunotherapies. By identifying distinct immune subtypes and their response profiles, one can move toward a future of personalized cancer immunotherapy, leading to better clinical outcomes for breast cancer patients.

Adaptive mechanisms in breast cancer evolution and drug resistance

In the malignant transformation of BC, two crucial features emerge metabolic reprogramming and immune evasion [62]. These characteristics promote the growth of cancer cells by enabling the acquisition of nutrients and energy and evading the body's immune system [63] (Fig. 3). Clinical trials have explored that patients with high mutational load or specific immune genes are more likely to receive immunotherapy [7]. On the other hand, tumors with low immunogenicity or tumors that develop immune evasion mechanisms often exhibit resistance to these treatments. Addressing to this heterogeneity requires a multiple approach, including developing biomarkers to predict response and combining immunotherapy with other treatments to improve their effectiveness [8].

Breast cancer and abnormal metabolism

BC, like many other malignancies, exhibits a hallmark feature known as metabolic reprogramming. This phenomenon involves a shift towards aerobic glycolysis, a process termed the "Warburg effect". BC cells exploit this pathway to generate lactate, which is subsequently released into the tumor microenvironment. Additionally, these cells exhibit an increased uptake of folate and acetate, facilitating rapid lipid biosynthesis. Furthermore, glutamine dependence serves as a protective mechanism against reactive oxygen species (ROS)-induced apoptosis (programmed cell death). These unique metabolic alterations represent promising avenues for the formulation of advanced and localized BC treatments [63].

BC cells exhibit a dysregulated metabolic profile, characterized by an upregulation of glucose metabolism as their primary energy source. This altered state involves several key pathways, including aerobic glycolysis, the tricarboxylic acid (TCA) cycle, the pentose phosphate pathway (PPP), and potentially gluconeogenesis [64].

Recent research highlights the growing recognition of aberrant lipid metabolism as a fundamental characteristic of cancer. Clinical data demonstrate a significant increase (20–40 %) in BC risk for postmenopausal obese women compared to their lean counterparts [65]. Beyond their roles in energy production and cellular membrane formation, lipids serve as essential signaling molecules within cells, acting

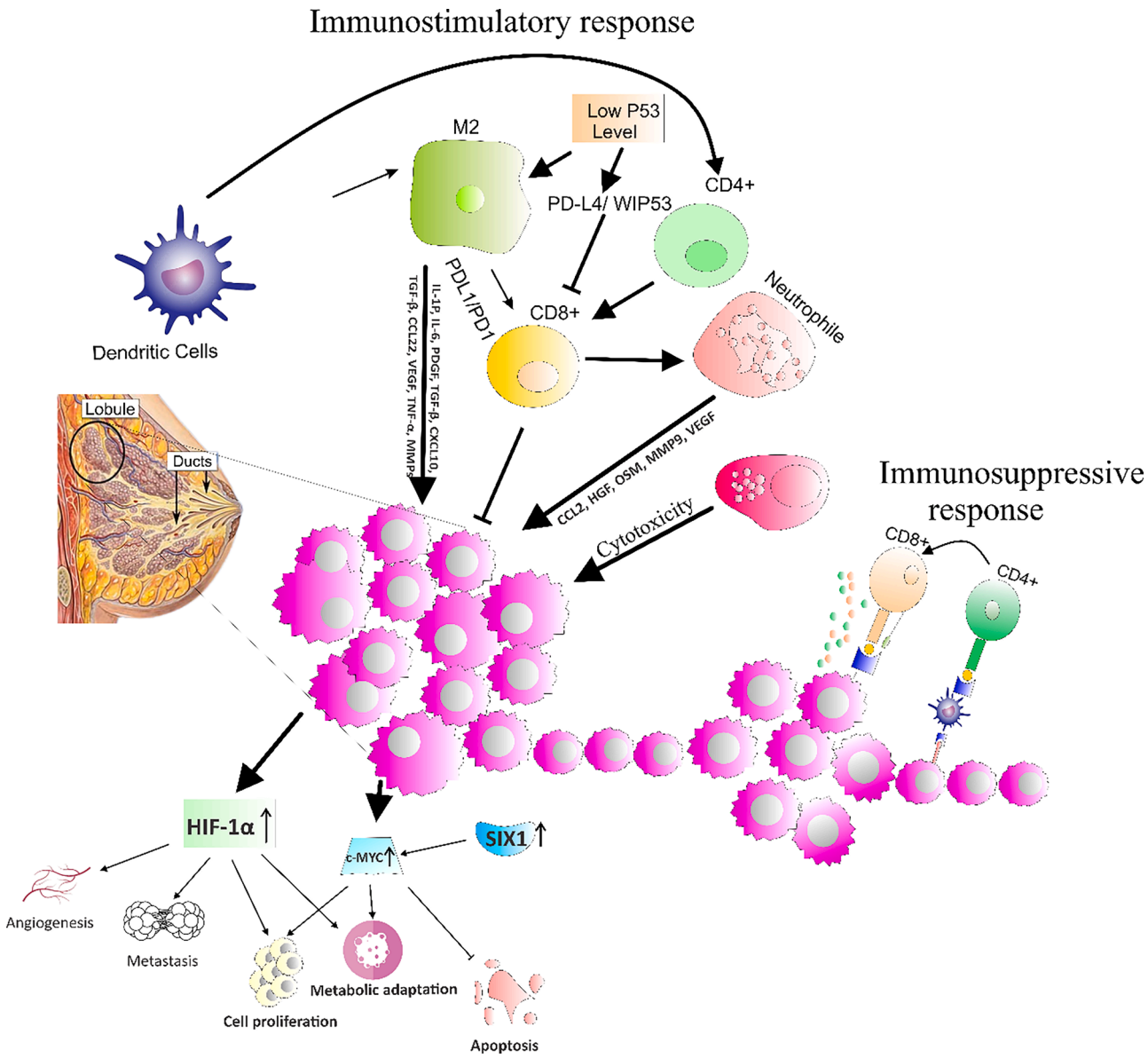

Fig. 3. Immunostimulatory and immunosuppressive response of tumor microenvironment complex and role of transcription factors in breast cancer progression.

as second messengers to regulate various cellular processes [66]. This additional function underscores the potential link between disrupted lipid metabolism and BC development.

The cancer cells exhibit an elevated demand for amino acids to fuel their rapid proliferation. These essential building blocks of proteins not only serve as structural components but also function as critical metabolites that regulate various aspects of cancer cell proliferation. It has been determined that fifteen amino acids have considerably higher concentrations than in normal samples making them potential hallmarks for early breast cancer diagnosis. In this research, particular attention was given to glutamine, serine, and glycine [67].

Primary transcription factors that govern lipid, proteins, and glucose metabolism

- **Hypoxia-inducible factor 1 (HIF1)**

Hypoxia, a state of limited oxygen availability, is recognized as a critical factor in BC development and progression [68]. In human BC patients, elevated expression of hypoxia-inducible factor 1α (HIF-1α) is a reliable indicator of adverse clinical results. HIFs, in particular HIF-1α, are essential for cells to be able to adapt to hypoxic conditions. HIF-1α expression is markedly elevated in TNBC, suggesting a possible function for it in this aggressive variety of BC [69] Fig. 3.

- **c-Myc**

The transcription factor c-Myc, which the Myc oncogene encodes, is overexpressed in between 30 and 50 percent of advanced breast tumors [70]. This overexpression may contribute to tumorigenesis and drug resistance because c-Myc regulates various cellular processes, including growth and metabolism [71]. Its dysregulation significantly contributes to BC development and progression, making c-Myc a promising target for novel therapeutic strategies.

- **SIX1**

SIX1, a homeobox transcription factor and the most well-characterized member of the SIX family, contributes significantly to the development of tumors, particularly breast cancer. It promotes

sustained proliferative signaling, a hallmark of cancer, by activating key cell cycle regulators cyclin A and D [72,73]. Notably, SIX1 overexpression is strongly associated with aggressive tumor behaviors in BC. These malignant properties encompass invasion, metastasis, the ability to evade growth suppressors, the transformation of healthy cells into cancerous ones, and resistance to cell death mechanisms [74]. This evidence highlights SIX1 as a potential target for therapeutic intervention in BC.

- **p53**

Mutant p53-expressing malignancies may rewire M2-type macrophages (M2), which promotes tumor invasion. High wild-type p53 activity acts as a brake on M1-like macrophages, reducing M1-like gene production. In response to stress, p53 activates programmed death-ligand 1 (PD-L1) and its receptor programmed death-1 (PD-1) in tumor cells and normal T cells, suppressing CD8+ *T* cells [75] (Fig. 3). p53 also plays a significant role in regulating glucose metabolism. It suppresses glycolysis and promotes oxidative phosphorylation. More specifically, p53 downregulates the genes of the GLUT family to prevent glucose uptake during aerobic glycolysis, which is responsible for glucose transport into cells. This dual function of p53 in modulating glucose metabolism adds to its complexity and relevance in cancer biology [76].

Improvements in breast cancer treatment

Recently, considerable, and in-depth studies have been conducted to gain a clearer insight into the mechanisms underlying abnormal metabolism in breast cancer. Concurrently, various targeted drugs have been discovered for the treatment of these metabolic abnormalities.

BC is frequently associated with obesity. Obese women are at higher risk of breast cancer than non-obese women. Hyperactivation of insulin-like growth factor-1 (IGF-1) signaling pathway partially supports this relationship. The IGF-1 system is essential for cell survival, proliferation, and differentiation and its dysregulation is correlated to various cancers, including BC [77]. MEDI-573 is a monoclonal antibody that inhibits IGF-1 binding to its receptor, IGF-1R offers an alternative treatment for this system. Clinical trials of phase 1 of BC patients treated with MEDI-573, showed better results which suggest it is an effective anticancer drug [78]. More research should be carried out to assess its long-term efficacy and safety in BC treatment. AMPK is a protein kinase with a dual role in the survival of cells in various circumstances.

Many drugs including fluoxetine, desmethoxycurcumin, and metformin overactivated the AMPK pathway to target BC cells and ultimately suppress the growth and development of BC [79].

Various inhibitors which target many metabolic pathways of BS have been discovered in the last decade. First-generation FASN inhibitors have severe side effects due to low cell permeability, while the newer inhibitor TVB-2640 is a potential inhibitor for clinical applications [80]. Gamma-glutamylcyclotransferase (GGCT) inhibitors like pro-N-glutaryl-l-alanine (pro-GA) have demonstrated inhibitory effects on cancer cell proliferation and migration [81]. Several other anticancer drugs targeting normal metabolism are in development, warranting further evaluation of their efficacy and potential side effects. Overall, these advancements in metabolic targeting offer exciting prospects for improving BC treatments.

Immune evasion in breast cancer

After the introduction of the concept of "immune surveillance" by Ehrlich in 1909, gradually, this theory evolved into the concept of "immunoediting," involving the three-phase process of cancer-immune system interaction: elimination, equilibrium, and escape [82]. Although immunotherapy has emerged as a revolutionary method of cancer treatment, its success rate in BC remains unsatisfactory. This limited efficacy is due to the complex interactions between the microenvironment of the tumor and cancer cells. The "escape phase" of the immunoediting process is very important since it describes how cancer cells resist immune system control. This escape refers to the formation of an immunosuppressive tumor microenvironment, which is characterized by decreased immune cell infiltration and function, as well as increased cancer cell proliferation [83]. After discussing the medical and biological difficulties, experts also discovered many possible ways to solve the problem. Increasing immunological response to targeting CTLA-4 and PD-1 and lymphocytic infiltration into the tumor microenvironment are potential methods to enhance the efficacy of BC immunotherapy [84,85].

Conclusion

Non-genetic heterogeneity, including epigenetic changes, cellular plasticity, and the tumor microenvironment, also determines cancer progression. In addition, such immune evasion decreases the effectiveness of new immunotherapies. Thus, a thorough approach is needed to address these problems. Non-genetic heterogeneity can be targeted with specific drugs that interrupt signaling pathways and transform the tumor microenvironment. With the use of immune checkpoint inhibitors, TIL therapy, and diverse combination immunotherapies, immune evasion could be targeted as well. Finally, different immune subtypes of BC could be identified due to the tumor immune microenvironment. The immune subtyping has a crucial effect on the prognosis, response to therapy, and clinical outcomes of the patients. Advancements in single-cell analysis and personalized medicine offer new opportunities for tailored treatments that can address the heterogeneity in breast tumors. However, there is a need to discover biomarkers that can predict specific therapy responses. Although advancements have been made in the identification of molecular targets, but not all patients with similar genetic variations respond similarly to treatments. So, there is a need to discover other factors responsible that may influence variations among such patients. Understanding mechanisms of resistance to existing therapies is another area that should be investigated to reduce reliance on alternative therapies and minimize economic loss. The future of BC treatment is hopeful, and continued progress leading towards better patient outcomes and a shift towards more specific and effective treatment strategies.

CRediT authorship contribution statement

Mudassir Hassan: Writing – review & editing, Writing – original draft. **Lütfi Tutar:** Writing – review & editing. **Duygu Sari-Ak:** Writing – review & editing, Writing – original draft. **Azhar Rasul:** Writing – review & editing. **Ejaz Basheer:** Writing – review & editing. **Yusuf Tutar:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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