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Review article

Heterogeneity of breast cancer: The importance of interaction between different tumor cell populations

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ABSTRACT

Introduction: Breast cancer is the most common cancer and the second leading cause of cancer-related death in women worldwide. Despite the early detection of breast cancer and increasing knowledge of its biology and chemo-resistance, metastatic breast cancer is largely incurable disease. We provide a review of the intertumor and intratumor heterogeneity, explain the differences between triple-negative breast cancer subtypes. Also, we describe the interaction of breast tumor cells with their microenvironment cells and explain how this interaction contributes to the tumor progression, metastasis formation and resistance to the treatment.

Discussion: One of the main causes that complicate the treatment is tumor heterogeneity. It is observed among patients (intertumor heterogeneity) and in each individual tumor (intratumor heterogeneity). In the case of intratumor heterogeneity, the tumor consists of different phenotypical cell populations. During breast cancer subtype identification, a small piece of solid tumor tissue does not necessarily represent all the tumor composition. Breast tumor cell phenotypical differences may appear due to cell localization in different tumor sites, unique response to the treatment, cell interaction with tumor microenvironment or tumor cell interaction with each other. This heterogeneity may lead to breast cancer aggressiveness and challenging treatment.

Conclusion: Understanding the molecular and cellular mechanisms of tumor heterogeneity that are relevant to the development of treatment resistance is a major area of research. Identification of differences between populations and their response to anticancer drugs would help to predict the potential resistance to chemotherapy and thus would help to select the most suitable anticancer treatment.

1. Introduction

Breast cancer is the most prevalent disease among women worldwide. Approximately 1.67 million new cases of this tumor were diagnosed in 2012 [1]. In 2018, new cases of breast cancer were diagnosed in nearly 2.1 million (11.6%) women, and about 627 thousand (6.6%) of women died from this type of cancer [2]. This prognosis suggests that several types of breast cancer are still incurable diseases leading to a high female mortality rate. Such aggressiveness of breast cancer may be due to the known heterogeneity of breast tumors [3]. One of the ways to determine cancer heterogeneity may be the identification of different cell phenotypes, cell density, or their localization in the tumor.

Heterogeneity typically exists between the similar type of tumors resulting in subtypes (intertumor heterogeneity), or within the tumors of the same type (intratumor heterogeneity). These specific subtypes are characterized by their molecular profiles, morphology, and expression of specific biomarkers (such as hormone estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 – HER2). For example, the part of cells that express the ER in breast tumors can alter widely, from 1 to 100% cells in the tumor [4].

Significant differences in individual tumors suggest that tumor cells can have various phenotypes with diverse functions and expression of different markers [4,5]. This intratumor heterogeneity is the tumor's ability to adapt to the new microenvironment conditions. Thus, tumor specimen taken during a biopsy does not necessarily represent the real tumor composition, because the tumor may consist of phenotypically different cancer cell populations with different properties and resistance to drugs. For this key reason, cancer treatment can be much more complicated [4].

Basal-like breast cancer subtype is considered to be one of the most aggressive ones and it is known as triple-negative breast cancer (TNBC) [6]. TNBC is characterized by reduced expression of hormone receptors.

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Currently, there is no molecular-based targeted therapy for TNBC. Therefore, TNBC is one of the highest priorities of current breast cancer research. More than 50% of patients diagnosed with TNBC at an early stage have a recurrence of the disease, and 37% of these patients die within the first 5 years, despite the treatment being applied [7]. The complicated treatment of TNBC can be associated with tumor heterogeneity. Nowadays, the TNBC classification into several different molecular TNBC subtypes is well known. Each molecular subtype has a different behavior of disease and response to treatment [8,9].

The variety of TNBC molecular subtypes proves the potential for heterogeneous tumors and may lead to further disease progression and treatment. The use of biological markers to identify subtypes of breast cancer has increased patient survival due to more accurate diagnosis of the disease. For example, when a breast cancer hormone receptor is detected, it is treated with endocrine therapy. HER2+ type tumors are usually treated with anti-HER2 therapy. Understanding breast cancer heterogeneity has become a significant achievement in identifying and treating breast cancer [10].

Therefore, it is very important to investigate breast cancer heterogeneity more thoroughly. Breast tumors can easily adapt to the unfavorable microenvironment, typically caused by standard chemotherapy or radiotherapy, remarkable lack of necessary oxygen, specific nutrients, etc. In this review, we focus on the interaction between phenotypically different cell populations and how it affects cancer development and resistance to chemotherapy.

2. Breast cancer heterogeneity

Intertumor heterogeneity typically describes key differences between tumors of the same origin in numerous patients (see Fig. 1a and b). These heterogeneous tumors have specific and individual molecular markers, unique biological behaviors and, as a result, different drug resistance and clinical outcomes [11]. Genetic mutations and/or epigenetic modifications are a source of intratumor heterogeneity. That explains why the same cell types have different phenotypic variants. Moreover, tumor microenvironment components, such as different populations of cancer fibroblast or stromal heterogeneity, immune system infiltration or dysregulation of the extracellular matrix, may cause different tumor variability [12,13].

Breast tumors are divided into several subtypes based on hormone receptor expression and the amount of cellular proliferation marker Ki67 (Fig. 2) [14]. Three molecular biomarkers ER, PR, and HER2 are used in the routine clinical management of patients with breast cancer. Differences in the expression of these receptors are named as biomarker heterogeneity [15]. ER-positive tumors are largely well-differentiated, less aggressive and associated with better outcomes after surgery [16]. PR-positive tumors usually are more aggressive than the ER-positive ones.

HER2 positive carcinomas are the most aggressive subtype in hormone receptors positive breast cancer group. However, these tumors give a satisfactory response to anti-HER2 targeted therapy [17]. Breast carcinomas that do not express ER, PR, HER2, are classified as TNBC. The reduced expression of hormone receptors makes more difficult to treat breast cancer since most hormone therapies target one of the three receptors, thus TNBC frequently requires combination therapies [18]. TNBC is a very heterogeneous cancer, considering the molecular and phenotypical features. Some subtypes of TNBC are known to be more aggressive, with a poor prognosis and feeble response to treatment, while other types have a better prognosis and good response to the treatment [18,19].

Moreover, scientists found that in some cases, breast cancer metastasis was formed with different molecular subtypes compared to the primary tumor molecular subtype. That was observed in luminal A tumor subtype which was converted to luminal B and HER-enriched one in more than half of the cases. Metastatic tissues show more significant expression of proliferative genes and poor expression of luminal-related genes compared to the primary tumor tissues. Basal-like tumors appear to be extremely stable from RNA-based analysis [20].

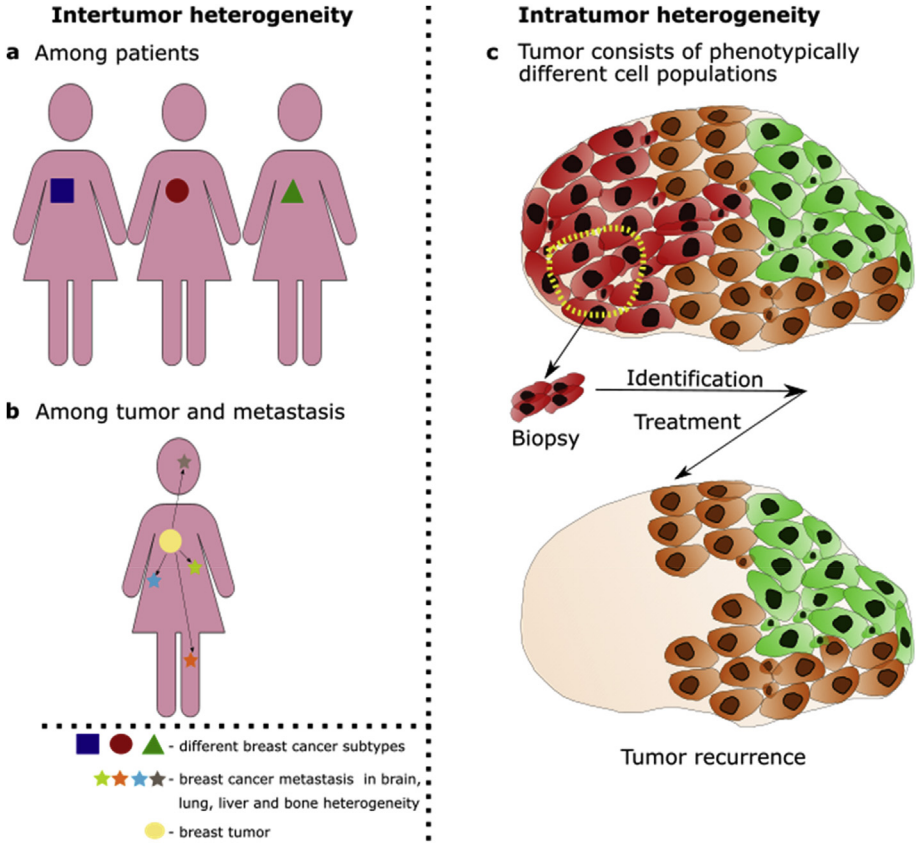

Fig. 1. Breast cancer intertumor and intratumor heterogeneity. **a** Variation of different breast cancer subtypes (based on receptor expression) among patients (intertumor heterogeneity). **b** Phenotypic differences of primary breast tumor and its metastasis in the same patient (intertumor heterogeneity). **c** Tumor subtype identification from a small part of tumor tissue with a "red" phenotype that is associated with a good prognosis may lead to not suitable treatment as it is lacking much more aggressive "green" and "brown" phenotype cells associated with a poor prognosis.). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

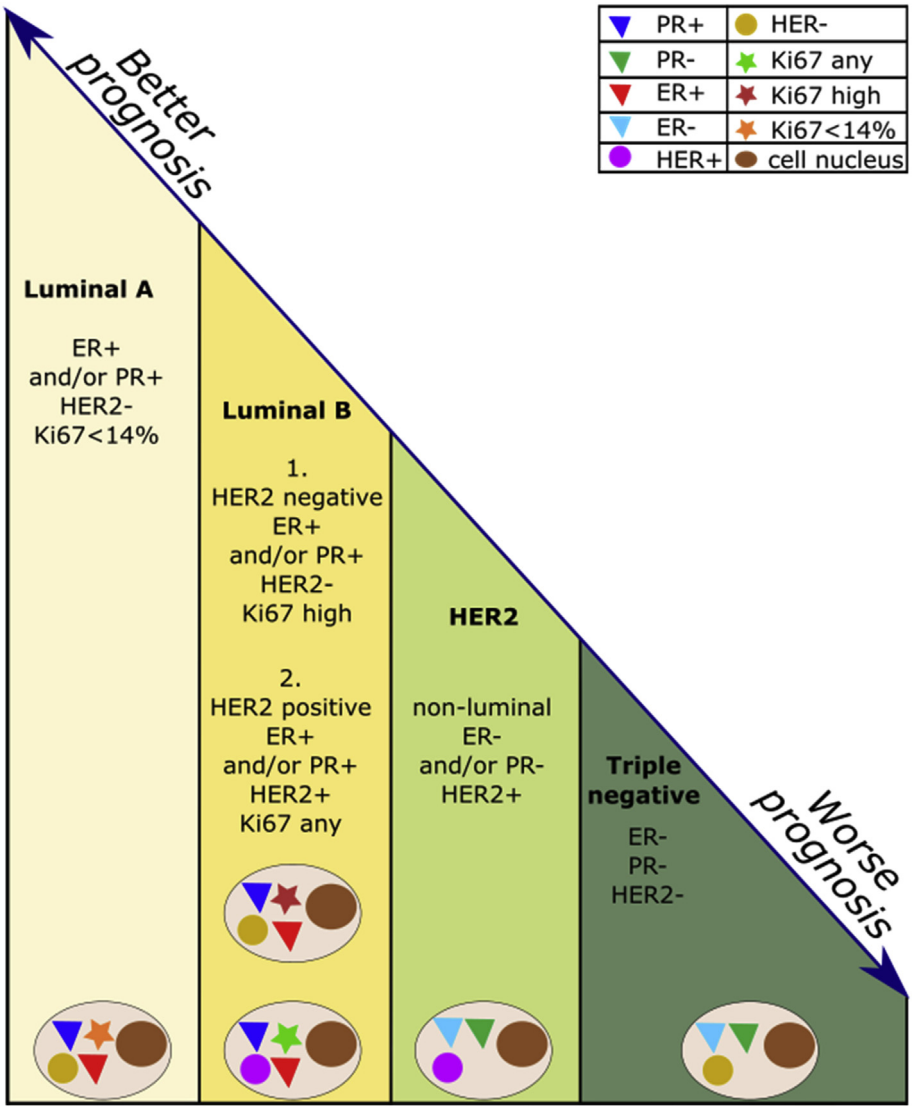

Fig. 2. Breast cancer classification based on hormone receptor expression.

The heterogeneity of breast tumors and their changes in disease relapse represent a formidable challenge of successful cancer treatment.

Individual tumor heterogeneity (Fig. 1c) has been observed about four decades ago in murine models [21] and it is named intratumor heterogeneity. Within individual cancers, there are distinct cancer cell subpopulations with the variable metastatic ability [21]. They represent differences for tumorigenicity, induction of senescence, activation of signalling pathways, migration, angiogenesis capacity, genetic signature, response to anticancer drugs [5,22]. Intratumor heterogeneity often leads to an unsuccessful diagnosis of the disease because tumor consists of phenotypically different cell populations and during tumor subtype identification, not all populations are identified. As a result, an applied treatment kills not all cell populations and the bulk part of the tumor could survive and lead to the disease relapse. Moreover, intratumor heterogeneity is a result of genetic mechanisms or environmental mechanisms, or both [23]. Many scientists believe that intratumor heterogeneity arises from cancer stem-like cells (CSCs) or by the clonal-evolution way [24,25].

Intertumor heterogeneity (breast cancer subtype) is determined by the molecular biomarkers identified in the tumor sample. In the clinic, breast cancer subtype usually is identified by immunohistochemical staining of solid tumor tissue taken during the biopsy. For example, if complete membrane staining is observed in more than 10% of tumor cells for HER2 receptor, it is considered as an invasive HER2 breast cancer subtype [26]. Although, ER, PR and AR status is considered negative if less than 1% of cells are stained positively by antibodies against these receptors [27]. It suggests that this tumor did not assign to ER or PR subtype and the tumor type is confirmed as HER2, but the tumor also contains several cells that express ER, PR receptors. Thus, treatment will be chosen for the bigger part of cells, in this case for HER2 and treated with trastuzumab or HER2-targeted therapy [28]. After such a treatment, HER2 subtype cells could be killed, but a few ER and PR-positive cells could survive, and it might cause a disease recurrence after several or maybe ten or more years. The deeper pathological diagnosis should be made for more advanced breast cancer diseases (invasive, metastatic or recurred disease), eg. by applyingmolecular methods such as *in situ* hybridization [29]. In this way, HER2 subtype will be considered positive if the number of HER2 gene copies is ≥ 6 or the ratio HER2/chromosome 17 is ≥ 2 [30]. However, tumor heterogeneity represents a critical barrier to identify the right tumor type and determine the appropriate treatment plan. Tumor heterogeneity is one of the major signs of malignancy [31].

Intratumor heterogeneity could be characterized not only by confirmation of the presence of different receptors but also by genome-wide tumor sequencing. Applying improved genome-wide sequencing technology, it is possible to define breast cancer subtypes based on

detailed copy-number variation (CNVs), DNA methylation, exome, RNA, microRNA sequencing and reverse-phase protein array data [32]. Solid tumor sample profiling can provide useful information about the tumor composition. However, it can not be applied for determination of cancer cell origin, topology or degree of intratumor heterogeneity [24]. Despite it, genome-wide studies of solid tumors still remain the most popular choice due to it's efficiency and relatively low price when compared to other methods.

Another one of an innovative methods to determine tumor composition is a single-cell sequencing. Analysis of a single cancer cell requires the isolation of some cancer cell populations based on phenotype, surface makers, etc The most important cell subpopulation that is isolated from solid tumors are primary tumor cells (PTCs), metastatic tumor cells (MTCs), cancer stem cells (CSCs), circulating tumor cells (CTCs) and disseminated tumor cells (DTCs). For cancer detection and treatment it is very important to understand the genetics and epigenetics features of cancer cell subpopulations. Tumor samples from biopsy or circulating tumor cells (CTC) in peripheral blood are the most objective way to assess intratumor heterogeneity and it allows to directly determine single cell genotypes. This allow to determine cancer cell origin, find differences between each cell in solid tumor, also evaluate their mutations and predict the potential drug resistance [33]. Single-cell sequencing method enables the identify not only the whole-genome mutations but also find the mutations that seem to evolve more progressively, generating large clonal diversity [33,34]. It means that single-cell analysis can help to identify and characterize clinically important subpopulations to develop successful treatment strategies [35,36].

Improve intratumor heterogeneity detection and characterization, CTCs isolation and analysis from body fluids are very important in cancer diagnosis and treatment. CTCs are cancer cells circulating in the peripheral blood that are could lead to cancer metastasis. Therefore, CTCs can be useful for diagnosis for therapeutic treatment, determination the risk of recurrence, choosing the right treatment scheme. But, the CTCs cells investigation is challenging process. These cells are very rare (low cell concentration in peripheral blood) and are very sensitive to traditional CTCs purification techniques. Scientists developed two effective methods for isolation of CTCs from body fluids. The first one is based on biomarkers anti-EpCAM-coated beads (EpCAM, epithelial cell adhesion molecule) and CTC-iChip magnetic bead system. This system leads to efficient fractionation of cells with only a few micromillimeters beads, and this results in a high yield and purity of isolated CTC [37]. CTC cell analysis usually is performed by several methods, such as quantitative Real-Time Polymerase Chain Reaction (qRT-PCR), fluorescence In Situ Hybridization (FISH) or Comparative Genomic Hybridization (CGH). These classical methods are used to determine important signatures of regulatory networks and biomarkers of CTC cells [38], detect and localize the presence or absence of specific DNA sequences on chromosomes [39], compare two genomic DNA samples arising from two sources, that are most often closely related (it is suspected that they contain differences in terms of either gains or losses of either whole chromosomes or subchromosomal regions) [40]. Thus, this CTCs detection and analysis methods are very helpful in the clinic for determining intratumor heterogeneity.

Heterogeneity has been observed not only in tumors. Ellsworth et al. [41] noticed heterogeneity in breast cancer cell lines among individual cells. The scientist explained that this might be related to DNA changes during cell division and cell-specific RNA variants that led to transcriptional heterogeneity and different response to drug-resistance and cell survival [41]. Moreover, scientists observed key differences between clones formed from single breast cancer cells. These clones demonstrated differences in their evolution, resistance to drugs, and it provided us with a better understanding of cancer behavior *in vitro* [27]. In another study, scientists showed that two single cells from luminal A and TNBC didn't display identical genome profiles [42].

Therefore, breast cancer heterogeneity exists in cell lines and in breast tumors. Investigation of tumor heterogeneity could have important implications for the diagnosis, therapeutic treatment and chemoresistance in breast cancer.

3. Clinical management of breast cancers based on intra- and/or intertumor heterogeneity

Knowledge and the clinical evaluation of breast tumor heterogeneity are of special importance in order to improve the patient treatment. The fast development of intertumor heterogeneity is not fit to the specific molecular classifications of breast tumors, which cause difficulties in clinical tumor identification and treatment [43]. The most complicated feature is intratumor heterogeneity as a temporal phenomenon different in each individual patient. This tumor "property" is closely related to cancer resistance to therapy, recurrence, and progression, and it is necessary to apply clinical methods directly to patient's material in today's clinical practice to be able to better define a specific effective treatment. For any breast cancer tumor subtype identification, we have only a few different molecular biomarkers that can be used in diagnostics at the moment and just a small part of drugs is suitable to apply in targeting therapy [44].

One of the most important issues is that breast cancer cells show different types of genetic instability (deletion, amplification, point-mutations and etc.) that lead to the high variability of cancer genomes, eg. promoting genetic heterogeneity and presents differences in treatment response [45]. Molecular and phenotypic abnormal variations are observed between tumors of different tissue, cell types, and also in the same tumor tissue within an individual patient [46]. Clinically, this heterogeneity of breast cancer can be categorized into three basic therapeutic groups:

(1) the ER-positive group. Patients with ER-positive tumor disease are receiving endocrine therapy [47], eg., tamoxifen, aromatase inhibitors [48];
(2) patients with TNBC; The effective treatment for this group is chemotherapy only. TNBC tumors are marked by the increased incidence of germline BRCA1 mutations [49]. TNBC therapeutic strategies include DNA repair complex targeting by platinum compounds and taxanes; targeting the P53 proteins with taxanes; inhibitrion of cells proliferation by using anthracycline-containing regimen (doxorubicin) [50];
(3) basal-like breast cancer group. It typically is characterized by the lack of expression of the molecular targets that confer responsiveness to highly effective targeted therapies, such as tamoxifen and aromatase inhibitors or trastuzumab [32].

Thus, the assignment of patients to a therapeutic group depends on the subtype of breast cancer and the stage of disease and aggressiveness.

However, highly specific and effective treatment for heterogeneous tumors is not applied. Based on the current knowledge of intratumor heterogeneity and tumor ability to change during treatment or progression, two strategies have been proposed to somewhat solve the problem. First strategy is to use the compounds targeting a shared pathway between different cancer cells in the same tumor [51]. It helps to affect more of breast cancer subtypes. For example, different molecular subtypes of breast cancer cells share pathways including Notch, Wnt, Her-2, and STAT3-NF-kB. It was observed, that the numerous mutations in breast cancer cells affect the same pathways, so agents that inhibits these pathways may bring the maximum benefit of precision therapy [52,53]. The second treatment strategy is to inhibit breast cancer cell plasticity (ability to change phenotypes) by interfering the cell shifting between cellular states. It could be done by inhibiting c-Met, TGF-beta [54,55] and PIK3CA genes, as their mutations in breast cancer cells might induce multipotency and cause intratumor heterogeneity [56].

Moreover, breast cancer inter-/intratumor heterogeneity has become a reason for medical and scientific researchers to seek the new types of clinical research approaches. Those studies, such as N1 trials (clinical trial in which a single patient is the entire trial, a single case study), umbrella (study multiple targeted therapies in the context of a single disease), basket (study a single targeted therapy in the context of multiple disease or disease subtypes), platform (study multiple targeted therapies in the context of a single disease in a perpetual manner, with therapies allowed to enter or leave the platform on the basis of a decision algorithm), have been proposed to overcome the limits of classical clinical research and to shorten the time for a wide clinical application [57,58].

Intratumor breast cancer heterogeneity causes difficulties in the treatment of this disease. Breast cancer solid tumors are composed of many different subpopulations, some which of them can be resistance to drugs used during treatment and can survive. While chemo-sensitive cells subpopulation was eliminated, chemo-resistant cells survive and become more fit in this new environment conditions. To solve it and to optimize chemotherapy to do it more efficient to avoid cell resistance, several methods have been developed, such as metronomic therapy and adaptive therapy. Metronomic therapy means that drugs are used scheduling of repetitive, low doses at regular intervals without longer interruption [59,60]. This therapeutic method is efficient in most breast cancer patients, but the effect of it on intratumor heterogeneity has not yet well studied [59]. Adaptive therapeutic strategy is therapy being evolved and changed in response to intratumor heterogeneity changes during the treatment period. The aim of adaptive therapy is to keep a stable chemo-sensitive cell population and inhibit the development of chemo-resistant population and thus increase the patient survival [61,62].

Despite the development of new drugs, clinicians and scientists expect that in the near future the better understanding of the relationship between intratumor heterogeneity and tumor response to therapy will possibly open a way to use already approved drugs in new treating schemes or combinations for a more effective personalized therapy.

4. Triple-negative breast cancer heterogeneity

TNBC is a breast cancer subtype defined by a lack of expression of hormonal receptors ER, PR, and HER-2. Thus the treatment of this disease is complicated, and it is characterized by a very poor prognosis following progression [63]. Although the TNBC displays a positive response to chemotherapy (anthracyclines or/and taxanes-based), early and higher rates of distant metastases (e.g. lung, brain, bones) and recurrences are observed [64]. TNBC heterogeneity is the main barrier in conquering breast cancer and thus it is important to focus more specifically on this disease. Historically, TNBC has been classified into six stable subtypes based on gene expression profiling by Lehmann B. et al. (Fig. 3). In 2011, TNBC was first divided into six molecular subtypes: basal-like 1 (BL1), characterized by increased cell cycle signalling and DNA damage response gene signature, high proliferative potential, and basal-like 2 (BL2), that showed high expression of growth factor receptors and high expression of myoepithelial marker subtypes. Moreover, they identified and characterized mesenchymal subtypes: mesenchymal (M) and mesenchymal stem-like (MSL), with high expression of specific genes associated with cell differentiation and growth factor signalling. The immunomodulatory (IM) subtype showed up with enriched immune cell processes and luminal androgen subtype (LAR) with increased expression of androgen signalling pathways [8].

In 2015, Burstein et al. proposed another classification (Fig. 3). TNBC was classified into four distinct subtypes: LAR, mesenchymal (MES), basal-like immune-suppressed (BLIS), and basal-like immune-activated (BLIA). Burstein was the first one to notice that the LAR subtype was identical to Lehmann LAR subtype. MES subtype included both the MSL and M subtype features according to Lehmann

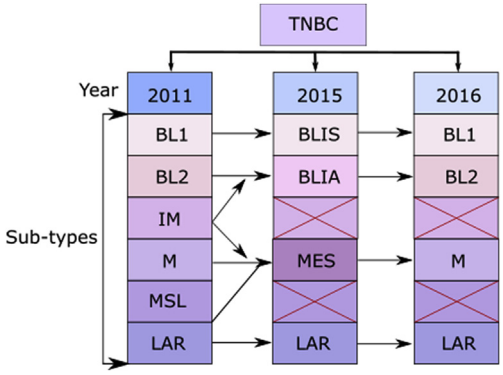

Fig. 3. Classification of triple-negative breast cancer subtypes based on gene expression profile. BL1, BL2 – basal-like 1,2; IM – immunomodulatory; M, MES – mesenchymal; MSL - mesenchymal stem-like; LAR - luminal androgen receptor; BLIS – basal-like immune-suppressed; BLIA – basal-like immune-activated; Black arrows indicate subtype regrouping over the year, red lines indicate subtypes that were not further excluded. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

classification. BL1 and BL2 subtypes have been attributed to BLIS and BLIA, while IM tumor subtypes were characterized as MES and BLIA [65].

LAR subtype is the most distinct from BLIS and BLIA subtypes. Patients diagnosed with BLIS breast cancer subtype have a very low survival, while the survival rates of patients with LAR subtype are the highest [66]. Furthermore, Burstein suggested several therapeutic agents to treat different TNBC subtypes. For example, LAR subtype could be treated with androgen receptor (AR) and surface mucin (MUC1) receptor blockers. MES subtype could be treated with platelet-derived growth factor (PDGF) receptor A and c-Kit inhibitors. BLIS subtype breast cancer could be treated by inhibiting subtype-specific targets, for example, inhibiting T cell activator V-set domain by VTCN1 (V-set domain-containing T-cell activation inhibitor 1) inhibitor. BLIA subtype cells growth could be stopped by targeting JAK-STAT signal transduction [9].

Lehman et al., in 2016 updated TNBC classification defining four tumor subtypes: BL1, BL2, M, and LAR. It was observed that patients with LAR subtype had increased local tumor spread and distant metastasis to the bones. Meanwhile, M subtype forms metastasis to the lung. These subtypes also differ by their response to chemotherapy. BL1 subtype usually is characterized by the lowest resistance to chemotherapy and the best survival rate after treatment [67].

Moreover, Herschkowitz et al., in 2007 discovered one more subtype – Claudin-low [69]. Several studies [70,74] have shown that Claudin-low tumor subtype could be assigned to TNBC but yet not classified, some scientist assigned this subtype to the basal-like group [71].

4.1. Basal-like subtype (BL)

BL tumors have been identified by BRCA1 gene mutation [72] and are characterized by rapid cell division, with an increased proliferation rate and loss of cell cycle control. BL subtype is described by low expression of the luminal and HER2 genes and high expression of basal genes. Enrichment of proliferation genes and increased Ki-67 expression suggest that this subtype would preferentially respond to antimitotic agents such as taxanes (paclitaxel or docetaxel) [73].

Based on gene expression, two BL subtypes have been identified: BL1 and BL2. BL1 subtype is typically characterized by many components associated with cell cycle and division pathways (DNA replication reactome, G_2 cell-cycle pathway, RNA polymerase, and G_1 to S cell cycle). The BL2 subtype is characterized by many growth factors

signalling pathways (EGF pathway, NGF pathway, MET pathway, Wnt/β-catenin, and IGF1R pathway) [8].

BL1 subtype is more sensitive to chemotherapy compared to the BL2 subtype. These subtypes have similar biology (high Ki-67 mRNA expression etc.) but different gene ontology: BL2 has specific gene ontologies (growth factor signalling - EGF, MET, and IGF-IR pathways). The difference in gene ontologies might explain the difference in chemosensitivity [74]. BL2 tumor could be targeted with EGFR or IGF1R inhibitors [74]. BL2 showed the highest resistance to chemotherapy.

4.2. Mesenchymal (M) and mesenchymal stem-like (MSL) subtype

The M and MSL subtypes share enrichment of genes for similar biological processes, involved in cell motility (actin regulation), ECM receptor interaction and cell differentiation pathways (Wnt pathway, anaplastic lymphoma kinase [ALK] pathway, and TGF-β signalling). It is important to note that M and MSL subtypes are significantly more sensitive to dasatinib compared to other TNBC subtypes [8]. Furthermore, M and MSL subtypes have morphological similarities: their cells are spindle-like when grown in monolayer, and stellate-like when grown in three-dimensional cell cultures [75].

However, M and MSL subtypes possess some unique properties. Growth factor signalling pathways and angiogenesis factors expression is increased in MSL subtype. The key difference between both subtypes is a reduced expression of proliferation genes in MSL subtype. It may be related to high expression of stem cells specific genes and markers. The MSL subtype that has a low expression of claudins genes (a family of proteins that are the most critical components of the tight junctions) is classified as to the Claudins-low breast cancer subtype and is described separately [8,67].

4.3. Immunomodulatory subtype (IM)

This breast cancer subtype is a histologically distinct and very rare form of TNBC [8]. IM subtype depends on many factors involved in immune cell processes like an immune cell signalling [76]. In IM subtype breast cancer cells has been observed increased activity of many pathways and receptors related to immune system: cytokine signalling (IL-12 and IL-7 pathway, cytokine pathway), immune signal transduction pathways (NFKB, TNF, and JAK/STAT signalling).

Currently, scientists identified that IM subtype could be treated with targeting anti-PD-1 and anti-PD-L1 agents [67]. In 2016, Burstein et al. [9] renamed IM subtype as MES and a basal-like immune-activated (BLIA) based on upregulation of genes that control B cell, immune T cell, and natural killer cell functions.

4.4. Luminal androgen receptor subtype (LAR)

The LAR subtype represents about 11% of all TNBCs and shows a poor response to standard chemotherapy [77]. Lehmann et al. identified differentially expressed hormone-regulated pathways of the AR in LAR subtype [8]. This subtype is characterized by a good prognosis as an effective treatment could be reached by using androgen receptor blockers. Increased gene expression of detoxification enzymes ALDH1A1 and ALDH3A1 can effectively inactivate some chemotherapeutic drugs like cyclophosphamide and cause the chemoresistance [78]. The second mechanism of the drug such as cisplatin and other alkylating agent's inactivation is increased activity of glutathione and glutathione-S-transferase (GST). The key step in the drug inactivation is a formation of conjugates between GSH and chemotherapeutics drugs where this process is catalysed by the GST [79]. These mechanisms are just a few of the many other drug inactivation mechanisms which can progressively eliminate chemotherapeutic drugs cytotoxicity effect of cancer cells and it causes chemoresistance. This group of tumors has delayed recurrences compared with the other groups and about 75% of distant metastasis occurred more than 3 years after disease diagnosis [74]. LAR subtype is the most resistant to chemotherapeutic drugs and is characterized by lower response to neoadjuvant chemotherapy in comparison to the other subtypes [67].

4.5. Claudin-low tumor subtype

Claudin-low subtype has been identified in 2007 by Herschkowitz et al. This subtype is characterized by low expression of genes involved in tight junctions and epithelial cell-cell adhesion, also the reduced expression of claudins 3, 4, 7 genes and E-cadherin gene [80]. Moreover, claudin-low tumor cells showed low expression of luminal epithelial genes and high expression of lymphocyte and endothelial cell markers [68]. These tumors are enriched in EMT and stem cell-like features while showing low expression of luminal and proliferation-associated genes. Acquisition of EMT or stem-like cell features is associated with the resistance to treatment and poor prognosis [81]. However, some chemotherapeutic drugs like sunitinib, regorafenib, and masitinib were effective in claudin-low tumors and inhibit cell migration, proliferation and metastasis formation [82].

5. Breast cancer phenotypic heterogeneity

Breast cancer classification based on immunohistochemical biomarkers is an important routine procedure to identify tumor subtype for the individual patient. However, sometimes breast cancer has features associated with different immunohistochemical phenotypes and it is impossible to assign it to specific breast cancer subtype. Breast cancer mixed phenotypes is a challenge to clinicians, especially when choosing the right targeting therapy [83].

Phenotypic heterogeneity became one of the most important research areas for scientists. Differences in cell morphology, proliferation rates, angiogenic and metastatic potential, and the expression of various surface markers are phenotypic heterogeneity features [84]. The phenotypic heterogeneity also can occur due to different cells localization in a tumor (tumor center or periphery, uneven oxygen amount), interaction with tumor microenvironment cells (fibroblast, adipocytes, macrophages and etc.) [85].

Recent studies present evidence of breast cancer heterogeneity in cell lines. Nguyen et al. [86] isolated clonal subpopulations from two breast cancer cell lines (MDA-MB-231 and CN34) and estimated intraclonal heterogeneities in these subpopulations. They isolated several subpopulations from parental MDA-MB-231 and CN34 breast cancer cell lines. One of the subpopulations was characterized as highly variable (HV) cell population in which increased ability to form metastasis in mice was observed. Scientists suggest that these subpopulation features are consistent with phenotypic diversification in cancer progression. In another study, several new cell sublines have been separated from parental MCF-7 cell line. Sublines were developed by incubating cells with tamoxifen, and later the sublines resistant to tamoxifen were selected. Several selected sublines acquired features characteristic to the triple-negative subtype [87].

Azzam and colleagues examined breast tumor-initiating stem cells (T-ISCs) in the cell line MDA-MB-231-luc and primary dissociated triple-negative breast tumor cultures (DT). It was found that in T-ISCs consists of phenotypically distinct subsets (subpopulations) based on CD44, CD24, ALDH, ESA expression [88].

Highly invasive MDA-MB-231 subpopulation has been characterized by Amaro et al. [89]. New subpopulations were named as invasive subpopulations (INV) and invasive subpopulation after long-term cultivation (LT). Both INV and LT subpopulations displayed significantly increased cell growth compared to parental MDA-MB-231 cell line. Cells from LT subpopulation were more aggressive and possessed different sensitivity to classic chemotherapeutic drugs compared to parental MDA-MB-231 cell line.

During tumor carcinogenesis, tumorigenesis and progression, individual cells may change and acquire new biological behaviours. This

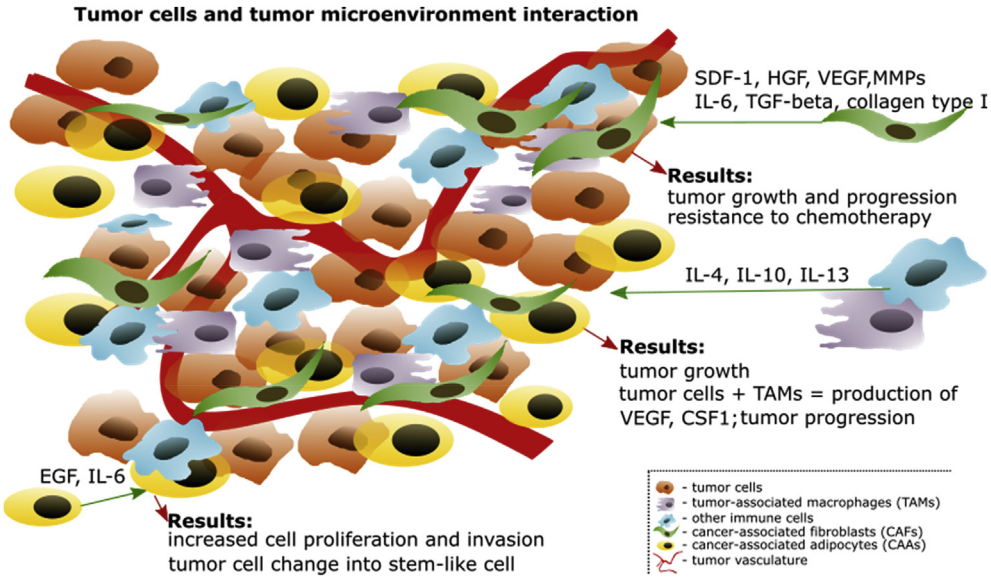

Fig. 4. Tumor cell interactions with the tumor microenvironment. Cancer-associated fibroblasts (CAFs) secrete many growth factors and it affects tumor growth. Interaction of immune cells, especially tumor-associated macrophages (TAMs), with tumor cells results in tumor growth, progression and secretion of specific factors. Adipocytes secreted factors increase tumor invasion and induce cell changes.

cellular heterogeneity in breast tumors could lead to higher aggressiveness and lower response to the treatment. Cancer stem cells (or tumor-initiating cells) can represent phenotypically different cancer cell populations and/or treatment-resistant cells. Cancer cells with stem cells properties may lead regrowth of tumor, and cells with drug resistance properties may give rise to cancer recurrence, despite a suitable therapeutic response [90].

6. Cell-cell interaction in breast cancer

Breast tumors are heterogeneous and consist of many different cell types. The heterogeneous population of stromal cells surrounds the tumor cells, and it creates tumor microenvironment (TME). Tumor development can influence its microenvironment and the microenvironment cells can affect tumor growth by secreted cytokines, growth factors, etc [91] (Fig. 4).

Nowadays researchers collect more and more evidences that TME is the key participant of tumor progression and response to the treatment. TME consists of components (normal stromal cells, immune cells, blood vessels, endothelial cells, adipocytes, etc.) that supply the necessary materials to tumor cells and it can affect tumor growth and progression [92]. TME stromal components are divided into several groups: cancer-associated fibroblasts, angiogenic vascular cells and infiltrating immune cells. These groups are very different in their function, but these cells may influence cancer cells growth, local invasion and metastasis [93]. Moreover, paracrine interaction between the stromal cell and tumor cells is the major mechanism by which stromal cells influence tumor cells and their behavior [94].

6.1. Cancer cells – fibroblast interaction

Fibroblasts are the major components of cancer stroma and are called cancer-associated fibroblasts (CAFs). CAFs is a cell type in TME that promotes tumor progression by initiating the remodelling of the extracellular matrix, secreting cytokines [95]. These cells play a significant role in tumor cell proliferation, invasion, and motility [92]. Conventional fibroblasts, not CAFs, are responsible for the extracellular environment by producing and remodelling the extracellular matrix (ECM). However, CAFs play a different role from normal fibroblasts and promote tumor progression [96]. CAFs stimulate tumor growth and progression by secretion stromal-derived factor SDF-1, which activates tumor cell proliferation via CXCR-4 receptor [97]. CAFs also secrete various factors: HGF, TGF-beta, VEGF, IL-6, etc. and matrix metalloproteinases (MMPs) in this way inducing stemness, epigenetic changes, EMT [98–100]. CAFs in the TME are responsible for the resistance to chemotherapy. Scientists found that CAFs secreted collagen type I is associated with the decrease of chemotherapeutic drug uptake [101]. It is shown that CAFs are also heterogeneous, like tumor cells. CAFs heterogeneity is related to their origin. Mao et al. characterized several CAFs types by their origin: 1) activated resident fibroblasts; 2) bone-marrow-derived mesenchymal stem cells; 3) cancer cells that undergo epithelial-mesenchymal transition; 4) other, unclassified [94].

Therefore, not only tumors cell interaction with CAFs could lead to tumor resistance to standard chemotherapy and disease progression, but CAFs heterogeneity may lead to different tumor progression and chemotherapy resistance levels.

6.2. Cancer cells – adipocytes interaction

Adipocytes are one more cell group that constitutes TME. Adipose tissue is the most plenty of component surrounding breast tumor cells. Cancer-associated adipocytes (CAAs) are tumor modifying cells and may cause a modification of cancer cell phenotype [102]. Adipocytes are producing hormones, growth factors, and cytokines. However, their role in breast cancer development is not clear yet [103]. Adipocyte interaction with breast cancer cells via IL-6 is associated with cancer cell phenotypical changes into stem cell phenotype [104]. Moreover, leptin and adiponectin produced by adipocytes increase cancer cell proliferation and invasion [105]. Therefore, factors secreted by adipocytes have an influence on tumor progression.

6.3. Cancer cells – immune/inflammatory cell interaction

The immune cells being in the breast tumors is normal tumor anatomy. Several types of immune cells are found in breast tumors: macrophages, T cells (various phenotypes), natural killer cells, B lymphocytes, mast cells, neutrophils [106]. Predominant immune cell type is macrophages derived from $CD34^{+}$ bone marrow progenitors. During a number of changes, these macrophages differentiate into tissue macrophages [107]. Macrophages in tumors are named as tumor-associated macrophages (TAMs). TAMs are immunosuppressive M2 polarized phenotype and secrete specific tumor cytokines (IL-4, IL-10, IL-13). These cytokines stimulate immune cell differentiation to mature macrophages and in such a way promote tumor growth [108]. The interaction between tumor cells and macrophages allow tumor cells to produce various factors, like VEGF, colony-stimulating factor–1 (CSF1),

and it also promotes tumor growth and invasion [109]. In breast cancer, the presence of infiltrating TAMs shows a poor disease prognosis [107]. Thus, immune cells, especially TAMs, may promote breast cancer progression.

Therefore, recently researchers have been concentrated on the study of tumor surrounding cells and their interaction with cancer cells. Growing understanding of cancer cell-cell interaction and cancer cell interaction with TME might be a powerful and useful tool in cancer prognosis and the development of new therapeutic drugs.

7. Conclusions

Breast tumor is a heterogeneous disease and displays various sensitivities to chemotherapy. Breast cancer subtypes variety and different classification variants show tumors heterogeneity. One of the most aggressive breast cancer subtypes is TNBC that is divided into seven molecular subtypes with different disease progression and aggressiveness. In one breast tumor could exist different types of cancer cells populations and this heterogeneity complicate correct identification of disease subtype. Many studies show that tumor cell interaction and their interaction with tumor microenvironment could have a significant influence on tumor progression and invasion. The ability to understand the influence of cell-cell interaction on tumor resistance would be a huge achievement in cancer treatment.

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