Review paper

Diagnostics and systemic treatment of triple-negative breast cancer: discoveries of the past, challenges for the future

Diagnostyka i leczenie systemowe potrójnie ujemnego raka piersi: dotychczasowe osiągnięcia i wyzwania przyszłości

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Słowa kluczowe: potrójnie ujemny rak piersi, podtypy molekularne, terapia personalizowana, inhibitory PARP, inhibitory PD-1.

Summary

Despite decades of progress in providing breast cancer patients with the most effective treatment methods, successful management of triple-negative subtype of this neoplasm remains an unmet need of oncology. Molecular heterogeneity, immunological evasion mechanisms and genomic instability all contribute to this cancer's troubling picture. Nevertheless, there have been substantial advances in exploration of the molecular landscape and innovative therapy of this entity. Discovery of molecular subtypes of triple-negative breast cancer has led to better understanding of the pathophysiological basis of the disease and gives hope for highly demanded personalized therapies. Work on targeted treatments is underway, including anti-Trop-2 antibodies, as well as PARP and PD-1/PD-L1 inhibitors. Efficacy of these drugs often relies on the presence of specific molecular targets; therefore there is a need for fast, affordable, and widely available molecular analysis methods. There is still a long road ahead, but with each discovery researchers are getting closer to making the diagnosis of triple-negative breast cancer less dire for the patient, with more effective tools at hand for the therapeutic team.

Streszczenie

Pomimo dekad rozwoju skutecznych metod leczenia raka piersi, terapia pacjentek z potrójnie ujemnym podtypem tego nowotworu wciąż w dużej mierze pozostaje niezaspokojoną potrzebą onkologii. Heterogeniczność molekularna, mechanizmy ucieczki immunologicznej i niestabilność genetyczna składają się na problematyczny obraz kliniczny nowotworu. Niemniej zachodzą istotne postępy w badaniach nad podłożem molekularnym oraz leczeniem tej choroby. Wyszczególnienie podtypów molekularnych potrójnie ujemnego raka piersi doprowadziło do lepszego zrozumienia patofizjologicznych podstaw jego rozwoju oraz daje nadzieję na rozwój terapii personalizowanych. Trwają prace nad leczeniem celowanym, w tym przeciwciałami anty-Trop2 oraz inhibitorami PARP i PD-1/PD-L1. Skuteczność tych leków często zależy od obecności konkretnych celów molekularnych, dlatego powstaje zapotrzebowanie na szybkie, niedrogie i szeroko dostępne metody badań molekularnych. Droga do celu jest długa, lecz każde odkrycie przybliża nas do punktu, w którym zespół terapeutyczny będzie dysponował skutecznymi narzędziami w walce z potrójnie ujemnym rakiem piersi.

Introduction

Breast cancer (BC) is the most commonly diagnosed malignancy in the world, as well as the leading cause of death from cancer in females [1]. Triple-negative breast cancer (TNBC) constitutes around 15–20% of all BC cases and is associated with the worst outcomes [2]. TNBC is immunohistochemically defined by lack of estrogen and progesterone receptors (ER, PR) as well as human epidermal growth factor receptor 2 (HER2) negativity [3]. Therefore, drugs that changed the landscape of BC, such as tamoxifen, aromatase inhibitors or trastuzumab serve no purpose in TNBC. Despite breakthroughs in tumor molecular analysis and the development of therapies such as poly (ADP-ribose) polymerase (PARP) inhibitors, programmed death-1/programmed death ligand-1 (PD-1/PD-L1) inhibitors, and anti-Trop-2 antibodies, managing this condition remains challenging due to molecular heterogeneity and the difficulty in identifying viable treatment targets. The purpose of this article is

to review the epidemiology of TNBC, its pathophysiological development, current treatment guidelines, and directions for further research.

Epidemiology

In spite of constantly developing methods of screening and treatment, BC remains the most common cause of cancer death in females worldwide. In 2020, it constituted 15.5% of all cancer deaths in females, and 6.8% of cancer deaths in both sexes [4]. In contrast to other subtypes of BC, TNBC is most common in younger, pre-menopausal women (average of 46.26 years old with median age of 62 years for all subtypes) [2]. 46% of patients with TNBC are expected to develop distant metastases and median survival among patients with metastatic TNBC is 13.3 months [5]. The pattern of metastasis differs from the one found in non-TNBC, which mostly tends to metastasize to bones, while TNBC is also likely to spread to the lungs and brain. The rate of relapse for TNBC is the highest among all the BC subtypes, at 70.2% over 5-year follow-up. Five-year relative survival is estimated at 76.9% (compared with 90% for all subtypes combined) [6]. Patients are most likely to die from TNBC during the first 4 to 5 years from diagnosis, with the peak for both metastases and deaths estimated at around 3–4 years. Therefore, for TNBC (similarly to HER-2 enriched subtype), the first 5 years of follow-up are crucial, while the frequency of further monitoring of the patient can be lower due to the steadily decreasing risk of relapse [6].

What is more, the COVID-19 pandemic has taken its toll as well. Its exact magnitude is yet to be determined, but countries that suffered the most during the outbreaks and failed to implement an efficient mechanism of prioritization in primary care and oncology are seeing significant rises in referrals of patients with advanced breast cancer. In early 2020, a 2-month delay in cancer referral in the UK, due to a nationwide hard lockdown, has been forecast to lead to 181–687 additional lives being lost due to BC over the next 10 years [7]. In Poland, based on a report by the National Institute of Oncology, significant decrease in the number of patients undergoing mammography, as well as treated with chemotherapy (CTx) or radiotherapy for BC, was observed in 2020 compared to 2019 [8]. Further, wide ranging analyses are therefore needed to assess the impact of the pandemic.

Developments in the understanding of TNBC

In 2000, breast tumors were characterized based on DNA microarray analysis by Perou *et al.* The researchers distinguished the following four major subtypes based on similarities in their "molecular portraits" (gene expression profiles): basal like, ErbB2 (HER2)-positive, normal breast-like and luminal epithelial/ER-positive [9]. Further research led to the subdivision of luminal subtypes [10]. Finally, in 2007, a complex molecular analysis of breast tumors performed by Herschkowitz *et al.* allowed for differentiation of subtypes based on expression of claudins, occludin, and E-cadherin [11]. "Claudin-low" tumors are now considered as a separate breast cancer phenotype, associated with poor prognosis [12]. Such a complex approach and thorough molecular analyses have facilitated research on treatment targets, as the basic histopathological division of tumors did not produce enough insight into therapeutic possibilities. Joint data from genome analysis and immunohistochemistry have provided a much more complete picture of the disease.

Among the discovered molecular subtypes, basal-like BC (BLBC) has been traditionally associated with triple-negativity, and as such, with the worst outcome and resistance to targeted treatment. However, now we know that although TNBC is an entity that often overlaps with BLBC (70–84% of TNBC are BL, and 70% of BLBC are TNBC), the terms must not be used as synonyms, and there are different genetic pathways leading to their development [13]. To clarify the matter, de Rujiter et al. introduced a classification which included three entities: non-triple-negative basal-like breast cancer (NT-BLBC), triple-negative basal-like breast cancer (TN-BLBC) and non-basal triple-negative breast cancer (NB-TNBC). Breast cancer 1 (*BRCA1*) mutation is the major causative factor of development of BLBC, while multiple other mutations may lead to loss of hormone receptors, destabilization of the genome and development of TN-BLBC [10].

Thorough analysis of gene expression of TNBC specimens has led to further subdivision of this entity into the following subtypes: basal-like 1 and 2 (BL1, BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL), and luminal androgen receptor (LAR), each associated with a unique combination of features, mutations and altered molecular pathways leading to development of the disease [3, 14, 15] (Table 1). Discovery of those 6 variants proved that TNBC is a heterogenous disease and has led to a focus on personalized therapies and more accurate monitoring of response to treatment. Over time, there has been a debate over whether the IM and MSL are true subtypes, or their identification was an effect of interference of the signal from infiltrating lymphocytes (for IM) and stromal cells (for MSL). Those subtypes were later removed from TNBC type-4 – the refined molecular classification by Lehmann *et al.* [16]. In 2015, Burstein *et al*. published another research paper concerning subtyping of TNBC. Their method of clustering identified four distinct subtypes: luminal androgen receptor, mesenchymal, basal-like immune-suppressed (BLIS; corresponding to BL1) and basal-like immune-activated (BLIA; corresponding to IM) [17]. Such observations

Table 1. Characteristics of 6 major molecular subtypes of triple-negative breast cancer

Subtype	Genetic background	Notable features, potential treatment targets
Basal-like 1	Rich in cell cycle, DNA replication, cell proliferation, division and repair genes	Elevated Ki-67 proliferation index. Highest rate of pCR. Highly susceptible to cisplatin containing CT regimens
Basal-like 2	Growth factor signaling: Wnt/beta-catenin, IGF1R, MET, EGF, NGF; glycolysis- and gluconeogenesis-related genes, myoepithelial markers	Low rates of pCR. Could be targeted with anti-EGFR, anti-VEGF agents and mTORi
Mesenchymal	Enriched in genes associated with cell migration pathways, differentiation pathways: Wnt, TGF-β	Due to tissue characteristics, prone to develop chemoresistance. Potential target of mTORi, EMTi
Mesenchymal stem-like*	Genes encoding EGFR, PDGF, VEGF; mesenchymal stem-cell associated genes	High rates of pCR. Potential target of anti-angiogenic therapies
Immunomodulatory*	High expression of genes encoding multiple factors partaking in immune processes, such as: PD-1, PD-L1, CTLA4, BIRC3, BTN3A1	Molecular picture overlapping with medullary breast cancer. High expression of PD-1, PD-L1 might suggest increased susceptibility to ICIs
Luminal androgen receptor	Steroid synthesis-related genes	Considered most resistant to CT. Anti-AR therapy recommended

**Later removed from TNBCtype-4 classification. pCR – complete pathological response, CT – chemotherapy, EGFR – epithelial growth factor receptor, VEGF – vascular endothelial growth factor, mTORi – mammalian target of rapamycin inhibitors, EMTi – epithelial-mesenchymal transition inhibitors, ICIs – immune checkpoint inhibitors, AR – androgen receptor.*

have been made possible thanks to increasing focus on the tumor microenvironment (TME) in oncology. In consequence, we acquired the possibility of extracting the intrinsic and extrinsic factors of tumor development and started to understand the bilateral relations of those factors in cancer biology. In 2012, researchers published an open-access online automatized subtyping tool for TNBC based on classification by Lehmann *et al.* (https://cbc.app.vumc.org/tnbc/).

In search of treatment targets and biomarkers

The set of mutations traditionally perceived as the source of genomic instability and poor prognosis in TNBC includes *TP53* (the most common mutation in TNBC), *PTEN*, *Rb* and *BRCA* alterations [14]. Other molecular abnormalities are under investigation as well, such as disruptions in phosphoinositide 3-kinase/AKT/mammalian target of rapamycin (PI3K/AKT/mTOR) and Kirsten rat sarcoma/Seven In Absentia Homolog/epidermal growth factor receptor (K-RAS/SIAH/EGFR) pathways [13].

The PI3K/AKT/mTOR pathway is a complex entity regulating, among other processes, cell growth, apoptosis, and even long-term potentiation of synapses. Overactivity of the pathway leading to unrestrained proliferation has been observed in various cancers [18]. The pathway is associated with phosphatase and tensin homolog (PTEN), which serves as its antagonist. Loss of this enzyme leads to PI3K/AKT/mTOR hyperactivity with all its consequences. Deregulation of PI3K/AKT/mTOR in various mechanisms is estimated to be present in 50% of TNBC [19]. Drugs targeting all three major components of the pathway are currently in clinical trials. Among them, inhibitors of AKT (AKTi) seem to be the most promising. Two major studies on efficacy of ipatasertib (LOTUS trial) and capivasertib (PAKT trial) as parts of the first-line TNBC treatment regimen both show significant improvements in progression-free survival (PFS) and overall survival (OS), with further improvements when targeting specifically patients with alterations in the PI3K/AKT/mTOR pathway or loss of PTEN [20].

While *KRAS* mutations have been linked to cancer development for decades, SIAH E3 ligase ($SIAH^{ON/OFF}$), the final "gatekeeper" in tumorigenesis of human cancer, is an emerging target in this oncogenic pathway. Early trials of SIAH proteolysis inhibitors have proven effective in hindering tumorigenesis in invasive breast cancer, lung cancer and pancreatic cancer [21]. Expression of EGFR and $SIAH^{ON/OFF}$ is also proposed as potential markers of response to treatment and prognosis in BC patients [22]. Anti-EGFR drugs, which are used with good results in various cancers, have not found use in TNBC yet, despite promising results of *in vitro* studies. Results of clinical trials for both monoclonal antibodies and tyrosine kinase inhibitors have been discouraging, with only a modest – if any – response [23]. Resistance to anti-EGFR antibodies in TNBC is a troubling matter – El Gurreab *et al.* hypothesized that the influence of the PI3K/AKT/mTOR pathway

is the reason for such a phenomenon and proposed a novel combination of agents – a mTOR inhibitor (everolimus) and gefitinib – and reported promising results of their *in vitro* studies [24].

Established treatment targets among the intrinsic factors of TNBC are *BRCA1* and *BRCA2* mutations and trophoblast cell-surface antigen (Trop2). In physiological conditions, the purpose of BRCA proteins is to repair damaged DNA. Mutations in the *BRCA* genes produce a faulty protein, unable to fulfil its function – DNA remains damaged, but partial repairs are still performed by other agents, allowing for replication of damaged genetic material and, consequently, cancer cell proliferation. One such enzyme is poly ADP-ribose polymerase (PARP). The mechanism of action of poly ADP-ribose polymerase inhibitors (PARPi) further hinders the repair of DNA damage, causing accumulation of a critical amount of errors, eventually leading to cell death. What is more, PARPi boost the DNA-damaging activity of platinum-based antineoplastics and radiation therapy – both commonly used in TNBC. OlympiAD – a major study on olaparib – has shown no significant improvement in OS compared to treatment of physician's choice (TPC), but highlighted the possibility of improving survival when used as first-line treatment, without prior CTx [25].

Trop2, first discovered by Lipinski *et al.* in 1981, is a transmembrane glycoprotein present in normal tissues and upregulated in most solid tumors [26]. Its presence has been detected in multiple oncogenic pathways. When activated, it sends a signal for cell proliferation, migration, self-renewal, and invasion. Its ubiquity has made Trop2 an ideal candidate for targeted treatment not only in TNBC, but also in numerous other neoplasms, such as small-cell lung cancer and pancreatic cancer [27]. Sacituzumab govitecan-hziy is an anti-Trop2 antibody conjugated with the topoisomerase inhibitor SN-38. Combined, they not only block activity of Trop2, but also disrupt DNA strands and lead to death of cancer cells. Promising results of the ASCENT clinical trial including improvement of overall response rates (ORR), OS and, most significantly, PFS of 5.6 months vs 1.7 months compared to TPC in metastatic TNBC have led to accelerated approval of the drug by the U.S. Food and Drug Administration.

A crucial example of the role of extrinsic factors in TNBC biology is the PD-1/PD-L1 inhibitory pathway. Increase of expression of PD-L1 on the surface of tumor cells increases their invasiveness, because activation of the PD-1/PD-L1 pathway leads to downregulation of activity of T-cells, T-cell lysis, decreased release of cytokines and increased tolerance of antigens [28]. Immune escape propagated by PD-L1 leads to larger tumor size, rapid growth, increased proliferation of cancer cells and higher grade [29]. While more common in ER-/HER2+ BC, overexpression of PD-L1 is estimated to be present in 20% of TNBC, which led to introduction of pembrolizumab and atezolizumab, anti-PD-1 and anti-PD-L1 antibodies, respectively, in treatment of this disease [30]. Efficacy of pembrolizumab plus CTx was investigated in the KEYNOTE-522 trial – the pathological complete response rate (pCR) was 64.8% vs. 51.2% in the placebo plus CTx group. Unfortunately, pembrolizumab significantly increased the rate of serious treatment-related adverse events (AE), with 23.3% of the patients discontinuing the therapy because of them [31]. Atezolizumab has been assessed in IMpassion trials. The IMpassion130 trial showed improvement in PFS of 7.2 months vs. 5.5 months for placebo plus albumin bound paclitaxel (nab-paclitaxel), slightly increased (7.5 vs. 5.0 months) for patients with confirmed PD-L1 positivity. OS was 21.3 months vs. 17.6 months, significantly increased when adjusted for PD-L1 positivity – 25.0 vs. 15.5 months. The discontinuation due to AE rate was lower than for pembrolizumab – 15.9% [32]. Recently published primary results of the IMpassion131 trial showed that combining atezolizumab with paclitaxel instead of nab-paclitaxel makes the treatment regimen inefficient. No significant differences in either OS or PFS between atezolizumab plus paclitaxel and placebo plus paclitaxel were observed. At the same time, 21% of patients discontinued the treatment due to AE [33].

Research has been conducted on various other targets. Studies on vascular endothelial growth factor (VEGF) inhibitors show some improvement in PFS, but no significant difference in OS, and/or long-term outcomes (LTO) in TNBC [34]. The reasons for such poor efficacy of VEGFi despite high expression of VEGF in TNBC cells are not clear. Considered targets also include: c-KIT/platelet-derived growth factor receptor A (PDGFRA), the rat sarcoma virus/mitogen-activated protein kinase (Ras/MAPK) pathway, Janus kinase 2 (JAK2), estrogen receptor β and long-chain fatty acyl-CoA synthetase 4 (ASCL4) [35].

Treatment of TNBC – current reality

The absence of classical treatment targets makes CTx the most used method of treatment, without many significant changes compared to regimens used in other aggressive subtypes of BC. The Polish Society of Clinical Oncology guidelines support application of multidrug neoadjuvant chemotherapy (NACT) in absence of contraindications. A regimen containing anthracyclines and taxanes is recommended for preoperative CTx, followed by carboplatin and docetaxel. Platinum-based agents are particularly beneficial for patients with *BRCA* mutations as they inactivate the faulty DNA strands, leading to death of the cancer cells. Another recommendation is application of dose-dense CTx (defined as shorter-interval CTx, with addition of granulocyte colony growth factor (G-CSF)) in high-risk populations [36].

The recent guidelines published by European Society for Medical Oncology also recommend sequential anthracyclines, taxanes and platinums in a neoadjuvant setting for early TNBC [37]. However, the 2021 guidelines for treatment of metastatic disease highlight the status of PD-L1 and *BRCA* as paramount factors, with addition of atezolizumab/pembrolizumab in PD-L1+ patients and PARPi in the *BRCA*-mutated population. Sacituzumab govitecan-hziy is recommended in the second line of treatment. The third line of treatment consists of eribulin, capecitabine or vinorelbine [38].

Application of NACT is supported by most researchers, as it not only leads to better outcomes in patients, but also allows for better stratification of patients in terms of risk and response to treatment, for instance by comparing pre- and post-NACT tumor sizes [22]. It is particularly important in TNBC, where workable markers are yet to be discovered. The Residual Cancer Burden (RCB) is a four-stage index developed by scientists from MD Anderson Cancer Center, the purpose of which is to evaluate residual disease after NACT. To calculate RCB, three numeric values concerning the primary tumor bed and three concerning lymph nodes are needed. RCB-0 indicates a complete pathological response (pCR), RCB-I stands for minimal residual disease, RCB-II for moderate residual disease and RCB-III for extensive residual disease. In 2020, Hamy *et al*. evaluated RCB in terms of its prognostic value in three subtypes of BC – luminal, HER2-positive and TNBC [39]. The index proved to be useful in prognosing which patients need a second line of treatment, due to elevated risk of recurrence (RCB-III). Therefore, calculating RCB should be highly advised in absence of stratification biomarkers.

Conclusions

Genomic instability, various pathways of immune escape, interaction of intrinsic and extrinsic factors resulting in chemoresistance, lack of universally proven methods of risk and response-to-treatment stratification – all these factors contribute to a challenging picture of triple negative breast cancer. Despite undeniable progress and breakthrough discoveries made in the 21st century, particularly following the first description of TNBC molecular subtypes, there are still not enough options at hand to offer patients faced with such a diagnosis. We still need to understand why certain targets are resistant to drugs used with success in other cancers, despite a similar "molecular picture." Another prerequisite needed to properly increase the efficacy of targeted treatment is widely available and affordable ways of molecular analysis – lots of research links the efficacy of novel drugs to high expression of specific targets. We also need to make up for arrears caused by the COVID-19 pandemic. Nevertheless, the progress does not stop here – increasing capabilities of molecular analysis, the multitude of clinical trials underway and adapting guidelines give hope for a brighter future for patients with TNBC.

Conflict of interest

The authors declare no conflict of interest.

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