Introduction

Breast cancer is one of the most common cancers in women and is a leading cause of cancer-related deaths. In 2018, ~266,000 women were expected to be diagnosed with invasive breast cancer in the United States, and 40,920 women were expected to die from it [1]. Triple-negative breast cancer (TNBC), which accounts for 15% of all breast cancers, is characterized by the lack of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression, which means that TNBC patients do not benefit from hormonal therapy or trastuzumab, which targets HER2 [2-4]. TNBC generally occurs in younger women and compared to other types of breast cancer, it shows a high histologic grade, a high propensity to metastasize to distant organs, and a poor outcome with a high recurrence rate after adjuvant therapy, which mainly consists of systemic cytotoxic chemotherapy [4]. Therefore, a significant challenge is to identify new targets and associated biomarkers for its treatment. Compared to other breast
cancer types, TNBC is characterized by a higher mutational load, which makes the tumor immunogenic and amenable to immunotherapeutic treatment [4]. Thanks to the promising results of immunotherapy in some cancers such as malignant melanoma and non-small cell lung cancer [5,6], several clinical trials are currently assessing immunotherapeutic approaches in TNBC patients [4,7–11]. The U.S. Food and Drug Administration (FDA) approval of nanoparticle albumin-bound paclitaxel (nab-paclitaxel) combined with atezolizumab in 2019 presented an innovative therapeutic development for TNBC patients. In this review, we discuss a brief history of TNBC classification based on gene expression patterns, as well as promising anticancer strategies for TNBC.

What Is TNBC?

In 1999, a molecular classification of breast cancer was first proposed by the National Cancer Institute (NCI) [12]. In 2000, Perou et al. [13] introduced a classification of breast cancer into four types: luminal, basal-like, HER2, and normal breast-like. The mammary epithelium consists of two layers: luminal cells and basal (myoepithelial) cells. The term “luminal” refers to the part containing mammary lobules and ductal structures, which are the major targets of estrogen and progesterone and mature to produce milk. In contrast, the term “basal” denotes another part of the mammary epithelium that supports the lobular and ductal structures. Sorlie et al. [14] further subdivided the luminal type into luminal A and B. The luminal A type demonstrated relatively high expression of the luminal epithelial gene cluster, including ER, GATA3, X-box binding protein 1, trefoil factor 3, hepatocyte nuclear factor 3a, FOXA1, and LIV-1. In contrast, the luminal B type showed high expression of proliferation-related genes and low to moderate expression of luminal-specific genes [14]. In a following study, breast cancer was classified into three major types based on the expression or absence of ER, PR, and HER2, namely ER+/PR+/HER2− (luminal A type), HER2+, and TNBC. The HER2+ type can be further divided into two subtypes: ER+/PR+/HER2+ (luminal B type) and ER−/PR−/HER2+ (HER2+ type) [15]. TNBC often occurs in under 40-year-old women and has a mortality rate of 40% within the first 5 years after diagnosis, a median survival time after metastasis of only 13.3 months, and a recurrence rate after surgery of 25%. Therefore, gene expression profiling analysis has begun to enable a deeper understanding of this disastrous disease, which is not sensitive to hormone therapy or HER2 treatment [2].

The Classification Begins!

Classification and analysis are milestones for scientific understanding. Now, it is TNBC’s turn. In 2011, Lehmann et al. [9] classified TNBC into six subtypes using 587 TNBC patients based on transcriptome profiling data as follows:

(1) The basal-like 1 subtype is involved in cell cycle and cell division pathways and shows overexpression of the AURKA, AURKB, BIRC5, BUB1, CENPA, CENPF, CCNA2, MYC, NRAS, PRC1, PLK1, and TTK genes. In addition, the expression of DNA repair (ATR/BRCA pathway)–related genes, such as CHEK1, FANCA, FANCG, RAD54BP, RADS1, NBN, EXO1, MSH2, MCM10, RAD21, and MDC1, is significantly increased.

(2) The basal-like 2 subtype is associated with increased expression of growth factor signaling pathways, including the EGF, NGF, MET, Wnt/β-catenin, and IGF1R pathways.

(3) The immunomodulatory subtype (IM subtype) shows high activation of immune signaling pathways (CTLA4, natural killer (NK)-cell, Th1/Th2, NFkB, TNF, T-cell, JAK/STAT, ATR/BRCA), and cytokine signaling pathways, such as the interleukin (IL)-12 and IL7 pathways.

(4) The mesenchymal subtype (M subtype) exhibits significantly lower expression levels in the immune signal transduction pathway, unlike the IM subtype. The M subtype also shows profound activation of cell migration-related signaling pathways, extracellular matrix receptor interaction pathways, and cell differentiation pathways, such as the Wnt pathway, anaplastic lymphoma kinase pathway, and transforming growth factor (TGF)-β pathway. These molecular changes result in sarcomatous morphological features.

(5) The mesenchymal stem-like subtype (MSL subtype) features high expression of stemness-related pathways, including the inositol phosphate metabolism pathway, G-protein-coupled receptor pathway, and calcium signaling pathway. In addition, the MSL subtype displays high expression of angiogenesis pathways such as KDR, TEK, TIE1, and EPAS1, but very low expression of the proliferative pathway. Moreover, this subtype is accompanied by high expression of stem cell markers (ABCA8, PROCN, ENG, ALDHA1, PERI, ABCB1, TERT2IP, and BCL2) and mesenchymal stem cell-specific markers (BMP2, ENG, ITGV, KDR, NGFR, NTSE, PDGFR, THY1, and VCAM1).

(6) The luminal androgen receptor subtype (LAR subtype) displays high expression of hormonal-related signaling pathways, including steroid synthesis, porphyrin metabolism, and an-
Subsequently, Burstein et al. [16] proposed four molecular subgroups using gene expression profiling of 198 TNBC cases as follows.

(1) The LAR subgroup is characterized by gene expression for hormone-related signaling pathways, including prolactin signaling and estrogen/androgen metabolism. The tumors within this subgroup show androgen receptor, ER, prolactin, and ErbB4 signaling, but are ERα-negative by immunohistochemistry (IHC) staining. ESR1 and other estrogen-regulated genes such as PGR, FOXA, XBPI, and GATA3 are expressed. This group demonstrates ER activation despite belonging to the category of ER-negative tumors by IHC, suggesting that traditional anti-estrogen therapies and anti-androgen therapies might be useful [9].

(2) The mesenchymal-like subtype is characterized by down-regulation of several signaling pathways, including cell cycle, mismatch repair, and hereditary breast carcinoma signaling pathways. In general, genes exclusive to osteocytes (OGN) and adipocytes (ADIPOQ, and PLIN1), and insulin-like growth factors (IGF-1) are highly expressed in this subgroup.

(3) The BLIS subtype, despite being ER-negative on IHC staining, shows activation of the ER signaling pathway. Steroid biosynthesis, porphyrin metabolism, androgen/estrogen metabolism, and peroxisome proliferator-activated receptor signaling pathways are highly activated in this subtype.

(4) The BLIS subtype is highly enriched in cell division and cell cycle-related signaling pathways, including DNA replication, DNA repair, mitotic cell cycle, mitotic prometaphase, and the M phase of the mitotic cell cycle. The BLIS subtype has high expression of proliferation-related genes, such as CENPF, BUB1, and PRC1, but this subtype is characterized by significant down-regulation of immune cell signaling pathways, immune response, and complement activation processes. The TNBC subtypes discussed above are summarized in Table 1.

**Immune Checkpoint Blockade Therapy in Certain TNBC Subtypes**

Targeting cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), programmed cell death protein 1 (PD-1), and its ligand (PD-L1) has revolutionized cancer treatment. CTLA-4 signaling is more prevalent in lymph nodes, and PD-1/PD-L1 is involved in multiple processes in the tumor microenvironment, such as suppression of T-cell responses and T-cell anergy. Blockade of PD-1/PD-L1 can significantly induce T-cell proliferation and activity, generating antitumor immune responses [6,18]. Thanks to this, immune checkpoint blockade (ICB) therapies have become a mainstay of treatment. According to Karn et al. [19], TNBC can be divided into immune-rich or immune-poor subtypes based on metagenes for a high lymphocyte infiltration (major histocompatibility complex class II) gene signature and low inflammation markers, such as interleukin-8 and vascular endothelial growth factor. Tumors with high immune gene expression had a better prognosis due to strict immunosurveillance and were associated with a low level of clonal heterogeneity, somatic copy number alteration level, mutations, and neoantigen load [19,20]. Of the TNBC subtypes, the IM and BLIA subtypes are classified as immune-rich.

CD8+ T cells are activated by antigen-presenting cells that present neoantigens on major histocompatibility complex class I and II
They are directly activated by their receptor and further regulated by a complex interplay of co-stimulatory and co-inhibitory signals, known as immune checkpoints [8].

Through these mechanisms, tumors can hijack physiological immune responses and cause immune tolerance. Sequential clinical trials on the treatment of TNBC with anti–PD-1/PD-L1 monoclonal antibodies (pembrolizumab and atezolizumab), significantly increased overall survival rates. In 2019, the U.S. FDA approved the use of nab-paclitaxel in combination with atezolizumab for PD-L1+ TNBC. At the moment, the primary challenge is to improve the response of patients with TNBC to anti–PD-1/PD-L1 treatment and to convert non-responders into responders.

What about the Microbiome?

Strong evidence from recent studies has suggested that the gut microbiota can affect the host’s antitumor immunity and that the composition of the intestinal microbiome may modulate the efficacy of ICB therapy in mice and humans [22-28]. Previous studies revealed that specific components of the gut microbiome might influence the efficacy of ICB therapy in patients, and primary resistance to ICB therapy could be overcome through treatment with ICB-promoting bacteria [29-33]. Notably, Mager et al. [30] demonstrated that *Bifidobacterium pseudolongum*, isolated from ICB-treated tumors, modulated enhanced ICB effects through production of the soluble metabolite inosine. Elevated systemic inosine levels and activated antitumor T cells are caused by the induction of decreased gut barrier function, resulting from ICB therapy in colorectal adenocarcinoma, urothelial carcinoma, and melanoma mice models. Inosine binding to T cell–specific adenosine 2A receptor (A2A) promotes Th1 cell activation [30]. Previous studies have demonstrated that inosine and A2A binding exert an inhibitory effect on Th1 differentiation *in vitro* and antitumor immunity *in vivo* [34-36]. Emerging studies have implicated the involvement of the gut microbiome in response to breast cancer treatment. Recently, in 2018, Banerjee et al. [22] discovered a predominant microbial signature in TNBC, which includes the families *Caulobacteriaceae*, *Actinomycetaceae*, *Enterobacteriaceae*, *Prevotellaceae*, *Sphingobacteriaceae*, *Brucellaceae*, *Flavobacteriaceae*, and *Bacilliaceae*. In conclusion, some types of gut microbiota and their metabolites are available to develop microbial-based adjuvant therapies that enhance the effectiveness of ICB therapy in TNBC patients (Fig. 1).

**Fig. 1.** Schematic relations among ICB therapies and gut microbiota. The well-established anticancer mechanisms of anti–PD-1, anti–PD-L1, and anti–CTLA-4 antibodies (bold text), as well-known ICB therapies, are illustrated; the efficacy of these therapies can be augmented by gut microbiota. The cytotoxic T-cell recognizing tumor antigen (red circle) causes TNBC cell death by blocking calm-down signaling using ICBs. Gut microbiota metabolites can strengthen the anticancer effects of anti–CTLA-4 antibody and present the tumor antigen to T-cells interacting with dendritic cells (upper right). ICB, immune checkpoint blockade; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; CTLA4, cytotoxic T-lymphocyte-associated antigen 4; TNBC, triple-negative breast cancer; TCR, T-cell receptor.
Table 1. Summary of the molecular classification of triple-negative breast cancer by gene expression

| Variable                      | Lehmann et al. (2011) [9] | Burstein et al. (2015) [16] | Liu et al. (2016) [17] |
|-------------------------------|---------------------------|-----------------------------|------------------------|
| Subtype/Dysregulated pathway  | 6 subtypes                | 4 subtypes                  | 4 subtypes             |
| Cell cycle                    | BL1                       | BLS                         | BLS                    |
| DNA repair                    | BL2                       |                             |                        |
| Immune signaling              | IM                        | BUA                         | IM                     |
| EMT signaling                 | M                         | MES                         | ML                     |
| Stemness-related signaling    | MSL                       | LAR                         | LAR                    |
| Hormone-related signaling     | LAR                       | Gene expression profile     | Gene expression profile |
| Modality                      | Gene expression profile   | copy number variation       | mRNA + lncRNA           |

BL1, basal-like 1; BLIS, basal-like immune-suppressed; BL2, basal-like 2; IM, immunomodulatory; BUA, basal-like immune-activated; M, mesenchymal; MES, mesenchymal; ML, mesenchymal-like; MSL, mesenchymal stem-like; LAR, luminal androgen receptor; lncRNA, long noncoding RNA.

Conclusion

TNBC is the most aggressive type of breast cancer and has a poor prognosis. Because it lacks the expression of hormone receptors and HER2 receptors, therapy is primarily based on systemic chemotherapy, rather than targeted agents. Although TNBC has a higher mutational load than other breast cancer types, it generally responds well to ICB therapy. This led to FDA approval for the use of nab-paclitaxel in combination with atezolizumab in TNBC patients. Thanks to new promising treatments such as ICB monotherapy and ICB therapy combined with conventional systemic chemotherapy, we currently face exciting new perspectives. As discussed above, many efforts are being made to increase the antitumor effects of ICB, and studies are intensively investigating the individual gut microbiota. Collectively, this research program will provide a profound understanding of TNBC pathology and insights into antitumor mechanisms, the first step in developing therapeutic strategies for this devastating type of breast cancer.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Gluz O, Liedtke C, Gottschalk N, Pusztai L, Nitz U, Harbeck N. Triple-negative breast cancer: current status and future directions. Ann Oncol 2009;20:1913-1927.
3. Hwang KT, Kim J, Jung J, Chang JH, Chai YJ, Oh SW, et al. Impact of breast cancer subtypes on prognosis of women with operable invasive breast cancer: a population-based study using SEER database. Clin Cancer Res 2019;25:1970-1979.
4. Yersal O, Barutca S. Biological subtypes of breast cancer: prognostic and therapeutic implications. World J Clin Oncol 2014;5:412-424.
5. Kitagawa S, Hakozaki T, Kitadai R, Hosomi Y. Switching administration of anti-PD-1 and anti-PD-L1 antibodies as immune checkpoint inhibitor rechallenge in individuals with advanced non-small cell lung cancer: case series and literature review. Thorac Cancer 2020;11:1927-1933.
6. Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, et
al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med 2015;372:320-330.

7. Fife BT, Bluestone JA. Control of peripheral T-cell tolerance and autoimmune via the CTLA-4 and PD-1 pathways. Immunol Rev 2008;224:166-182.

8. Jung K, Choi I. Emerging co-signaling networks in T cell immune regulation. Immune Netw 2013;13:184-193.

9. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 2011;121:2750-2767.

10. Pentcheva-Hoang T, Corse E, Allison JP. Negative regulators of T-cell activation: potential targets for therapeutic intervention in cancer, autoimmune disease, and persistent infections. Immunol Rev 2009;229:67-87.

11. Sharpe AH, Wherry EJ, Ahmed R, Freeman GJ. The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. Nat Immunol 2007;8:239-245.

12. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci U S A 1998;95:14863-14868.

13. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature 2000;406:747-752.

14. Sorlie T, Perou CM, Tibshirani R, Aas T, Geiser S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001;98:10869-10874.

15. Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A 2003;100:8418-8423.

16. Burstein MD, Tsimelzon A, Poage GM, Covington KR, Contreas A, Fuquaa SA, et al. Comprehensive genomic analysis identifies novel subtypes and targets of triple-negative breast cancer. Clin Cancer Res 2015;21:1688-1698.

17. Liu YR, Jiang YZ, Xu XE, Yu KD, Jin X, Hu X, et al. Comprehensive transcriptome analysis identifies novel molecular subtypes and subtype-specific RNAs of triple-negative breast cancer. Breast Cancer Res 2016;18:33.

18. Polk A, Svane IM, Andersson M, Nielsen D. Checkpoint inhibitors in breast cancer: current status. Cancer Treat Rev 2018;63:122-134.

19. Karn T, Jiang T, Hatzis C, Sanger N, El-Balat A, Rody A, et al. Association between genomic metrics and immune infiltration in triple-negative breast cancer. JAMA Oncol 2017;3:1707-1711.

20. Michel LL, von Au A, Mavratzas A, Smetanay K, Schutz F, Schneeweiss A. Immune checkpoint blockade in patients with triple-negative breast cancer. Target Oncol 2020;15:415-428.

21. Zhang X, Kim S, Hundal J, Herndon JM, Li S, Petti AA, et al. Breast cancer neoantigens can induce CD8(+) T-cell responses and antitumor immunity. Cancer Immunol Immunother 2017;5:516-523.

22. Banerjee S, Tian T, Wei Z, Shih N, Feldman MD, Peck KN, et al. Distinct microbial signatures associated with different breast cancer types. Front Microbiol 2018;9:951.

23. Fuhrman BJ, Feigelson HS, Flores R, Gail MH, Xu X, Ravel J, et al. Associations of the fecal microbiome with urinary estrogens and estrogen metabolites in postmenopausal women. J Clin Endocrinol Metab 2014;99:4632-4640.

24. Goedert JJ, Hua X, Bielecka A, Okayasu I, Milne GL, Jones GS, et al. Postmenopausal breast cancer and oestrogen associations with the IgA-coated and IgA-noncoated faecal microbiota. Br J Cancer 2018;118:471-479.

25. Hieken TJ, Chen J, Hoskin TL, Walther-Antonio M, Johnson S, Ramaker S, et al. The microbiome of aseptically collected human breast tissue in benign and malignant disease. Sci Rep 2016;6:30751.

26. Luu TH, Michel C, Bard JM, Dravet F, Nazih H, Bobin-Dubigeon C. Intestinal proportion of Blautia sp. is associated with clinical stage and histoprognostic grade in patients with early-stage breast cancer. Nutr Cancer 2017;69:267-275.

27. Urbaniaik C, Gloor GB, Brackstone M, Scott L, Tangney M, Reid G. The microbiota of breast tissue and its association with breast cancer. Appl Environ Microbiol 2016;82:5039-5048.

28. Vezzoni M, Pitt JM, Dailiere R, Lepage J, Waldschmitt N, Flament C, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science 2015;350:1079-1084.

29. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpintets TV, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science 2018;359:97-103.

30. Mager LF, Burkhardt R, Pett N, Cooke NC, Brown K, Ramay H, et al. Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. Science 2020;369:1481-1489.

31. Matson V, Fessler J, Bao R, Chongsuwat T, Zha Y, Alegre ML, et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. Science 2018;359:104-108.

32. Routy B, Le Chatelier E, Derosa L, Duong CP, Alou MT, Dailiere R, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science 2018;359:91-97.

33. Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science 2015;
34. Hasko G, Kuhel DG, Nemeth ZH, Mabley JG, Stachlewitz RF, Virag L, et al. Inosine inhibits inflammatory cytokine production by a posttranscriptional mechanism and protects against endotoxin-induced shock. J Immunol 2000;164:1013-1019.

35. He B, Hoang TK, Wang T, Ferris M, Taylor CM, Tian X, et al. Resetting microbiota by Lactobacillus reuteri inhibits Treg deficiency-induced autoimmunity via adenosine A2A receptors. J Exp Med 2017;214:107-123.

36. Csoka B, Himer L, Selmeczy Z, Vizi ES, Pacher P, Ledent C, et al. Adenosine A2A receptor activation inhibits T helper 1 and T helper 2 cell development and effector function. FASEB J 2008; 22:3491-3499.