REVIEW

Triple-negative breast cancer and basal-like subtype : Pathology and targeted therapy

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Abstract : Triple-negative breast cancer (TNBC) is a heterogenous disease. For personalized medicine, it is essential to identify and classify tumor subtypes to develop effective therapeutic strategies. Although gene expression profiling has identified several TNBC subtypes, classification of these tumors remains complex. Most TNBCs exhibit an aggressive phenotype, but some rare types have a favorable clinical course. In this review, we summarize the classification and characteristics related to the various TNBC subtypes, including the rare types. Therapeutic methods that are suitable for each subtype are also discussed. Of the intrinsic breast cancer subtypes identified by gene expression analysis, the basal-like subtype specifically displayed decreased expression of an estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2) cluster. We also present results that characterize the TNBC and basal-like phenotypes. TNBC may be categorized into four major classes : basal-like, immune-enriched, mesenchymal, and luminal androgen receptor. Therapeutic strategies for each subtype have been proposed along with newly approved targeted therapies for TNBC, such as immune checkpoint inhibitors. Understanding the classification of TNBC based on gene expression profiling in association with clinicopathological factors will facilitate accurate pathological diagnosis and effective treatment selection. J. Med. Invest. 68 : 213-219, August, 2021

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INTRODUCTION

The biological indicators of breast cancer include tumor invasion diameter, number of metastatic lymph nodes, disease stage, histological grade, nuclear grade, histological type, and lymphatic/vascular invasion (1, 2). The estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2), and Ki-67 have been evaluated in pathological specimens using immunohistochemistry (IHC) and fluorescence *in situ* hybridization. Significant attention has been focused on the classification of breast cancer based on gene expression profiling ; however, IHC has the advantage of cost and time compared with microarray analysis and can also reveal local expression including *in situ* lesions and invasive lesions. For routine clinical practice, the immunohistochemical classification of breast cancer is used as a surrogate for subtype and a guide for treatment selection (3, 4).

Triple-negative breast cancer (TNBC) is devoid of hormone receptor (ER and PgR) expression, as well as overexpression or amplification of HER2. Both hormone and anti-HER2 therapies are ineffective for TNBC ; therefore, chemotherapy is required. TNBC represents a variety of tumors, and ongoing research is aimed at developing targeted treatments based on its biological characteristics (5). Cancer genomic panels using formalin-fixed, paraffin-embedded tumor blocks have enabled the examination of many important genes and biomarkers that may indicate if a targeted therapy or a clinical trial is warranted for a patient (6).

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TNBCs can be subtyped by various molecular classification schemes. The basal-like subtype is a type of invasive breast cancer originally defined by gene expression profiling studies. Protein expression patterns characteristic of the basal-like subtype include high expression of basal keratins and epidermal growth factor receptor (EGFR) (7). The basal-like subtype exhibits pathological features including high histological grade, high proliferative rate, and prominent lymphocyte infiltration (8, 9) and is enriched in patients harboring tumor suppressor protein 53 (*TP53*) and *BRCA1* mutations (10, 11). Most basal-like subtype breast cancers exhibit poor prognosis (12). However, some rare TNBC types have exceptionally good prognosis. Basal-like subtype breast cancer and TNBC share common biological and pathological features with a 70%–80% overlap (13).

This review focuses on the classification of TNBC subtypes compared with the histology of the basal-like subtype and other rare subtypes. We also discuss targeted therapies that are available for each TNBC subtype and introduce some of our study results, including characteristics of TNBC and the basal-like phenotype.

TNBC SUBTYPING

Gene expression profiles and classification of the intrinsic TNBC subtypes

Breast cancer is a heterogenous group of diseases that can be categorized using gene expression profiling into luminal A, luminal B, HER2-enriched, basal-like, and normal-like subtypes (14). These so-called intrinsic subtypes show differences in incidence, age at diagnosis, prognosis, and response to treatment (14, 15). Lehmann *et al*. identified six TNBC subtypes using the gene expression profiling results of 21 breast cancer datasets. These included the basal-like subtype, which was divided into

BL1 and BL2, and four other subtypes : immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL), and luminal androgen receptor (LAR). For BL1, pathways associated with cell division and DNA damage repair (ATR/BRCA) were enriched, and Ki-67 expression was high, suggesting active cell proliferation. For BL2, growth factor signaling (EGF, NGF, MET, Wnt/β-catenin, and IGF1R pathways), glycolysis, and gluconeogenesis-related genes were upregulated. Growth factor receptors and myoepithelial markers (TP63, CD10) were also highly expressed (16). A histopathological examination and gene expression analysis of laser-capture microdissection specimens revealed that the IM type contained infiltrating lymphocytes and the MSL type contained many stromal cells in the tumor tissue. The classification was organized into four subtypes : BL1, BL2, M, and LAR (TNBC type-4) (17).

The claudin-low subtype was considered to be a new intrinsic subtype. This subtype was characterized by low or absent expression of luminal differentiation markers and tight junction proteins, as well as enrichment of epithelial-mesenchymal transition markers, immune response genes, and cancer stem cell-like features (18). Most claudin-low subtypes showed M and MSL subtypes as classified by Lehmann *et al*. (19).

Burstein *et al*. classified TNBCs into four types using RNA and DNA profiling : basal-like immune-suppressed (BLIS), basal-like immune-activated (BLIA), mesenchymal (MES), and luminal/androgen receptor (LAR). The expression levels of genes controlling B cell, T cell, and natural killer cell functions were low in the BLIS subtype and high in the BLIA subtype. In the BLIS subtype, the expression of molecules involved in antigen presentation, immune cell differentiation, and signal communication between immune cells was low ; however, the expression of SOX family transcription factors was high. The BLIA subtype exhibited significant STAT transcription factor-mediated pathway activation. Among the four subtypes, BLIA had the best prognosis, whereas BLIS had the worst (20).

The classification of TNBC is very complex and involves basal markers, stem-cell markers, mesenchymal traits, androgen receptor (AR) expression, and immune markers (21).

TNBC and basal-like subtype, surrogate subtype classification by immunohistochemistry

High molecular weight cytokeratins, such as CK5/6, CK14, and CK17, are expressed in cells near the basement membrane in many epithelia and are known as basal keratins (22). IHC analysis of the basal keratins is used to define breast cancer exhibiting a basal phenotype as a surrogate of gene expression.

Breast cancer with a basal phenotype is common in young people and is characterized by histologically prominent mitotic figures and nuclear atypia. It is also accompanied by extensive necrosis and marked lymphocyte infiltration (8, 9). The majority of breast cancers that occur in *BRCA1* mutation carriers are of the basal-like subtype (11). Tumors arising in *BRCA1* carriers have many similarities to sporadic basal-like tumors with *BRCA1* dysfunction, such as low expression of *BRCA1* messenger RNA (23).

In our study, TNBC accounted for 15% of the 513 invasive cancers examined. Among the TNBCs, 61% were positive for CK5/6, CK14, or CK17 by IHC and exhibited a basal phenotype. Among the TNBCs, those exhibiting a basal phenotype had a larger tumor diameter, higher nuclear grade, and higher EGFR positivity compared with the non-basal phenotypes. In addition, breast cancer exhibiting a basal phenotype was not significantly different from the non-basal phenotype within TNBC, although there was a tendency for early recurrence (24).

The basal-like subtype has much in common with TNBC histology and clinical behavior, but it is not exactly the same. It has been reported that 21% of TNBCs are not basal-like and 31% of breast cancers showing basal-like gene expression are not triple-negative (13).

There is currently no consensus on the optimal immunohistochemical panel to define the basal-like subtype. At the 2017 St. Gallen Consensus Conference, it was determined that the basal-like subtype should be defined by gene expression profiling assay only (4) (Figure 1). In the latest WHO classification of breast cancer, the basal-like subtype was recognized as one of the intrinsic breast cancer subtypes based on genetic analysis like a quantitative RT-PCR-based test with a curated list of 50 genes (the PAM50 gene signature) (25). According to the WHO classification, breast cancers showing basal-like features are described as carcinomas showing a medullary pattern, which is one of the special morphological patterns of invasive breast carcinoma of no special type (26).

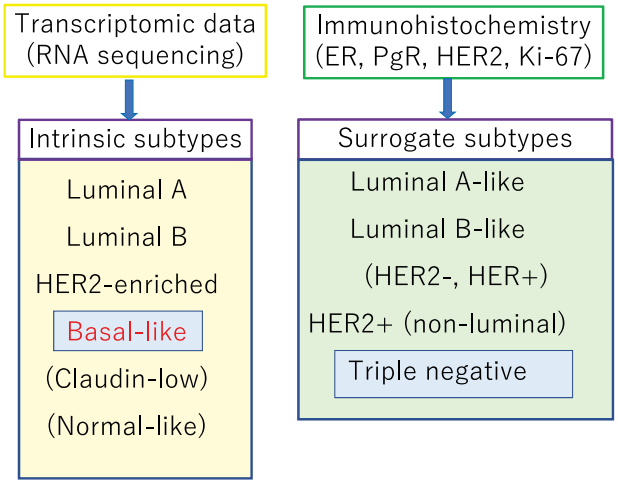

Figure 1. Surrogate subtypes with immunohistochemistry and intrinsic subtypes of breast cancer. Modified from Fig.2.88 in WHO classification of tumours, 5th edition, breast tumours.

HISTOLOGICAL TYPES OF TNBC

Most TNBCs are poorly differentiated, highly malignant invasive ductal carcinomas (8, 9). However, TNBC is a heterogeneous disease and includes special morphological patterns and rare types that include carcinoma with medullary pattern, apocrine carcinoma, adenoid cystic carcinoma (ACC), secretory carcinoma, and metaplastic carcinoma (Figure 2A-H).

Carcinoma with medullary pattern is often associated with significant lymphocyte infiltration and has a good prognosis despite its high-grade histology (27). Carcinoma with medullary pattern is correlated with the intrinsic IM subtype as classified by Lehmann *et al*. (16) and a subgroup of the basal-like subtype (28).

Carcinoma with apocrine differentiation is characterized by increased androgen signaling and often overlaps with the HER2 group based on their gene expression profiles (29).

ACC is a salivary gland-type breast cancer that is generally low-grade and shows an indolent clinical behavior (30). Approximately half of ACCs are found in the subareolar area. The presence of a *MYB-NFIB* fusion gene resulting from a specific t(6 ; 9) chromosomal translocation was found in ACC (31).

Secretory carcinoma is associated with a t(12 ; 15) translocation, which results in an *ETV6-NTRK3* fusion gene (32). Most secretory carcinomas exhibit low to moderate grade histology with

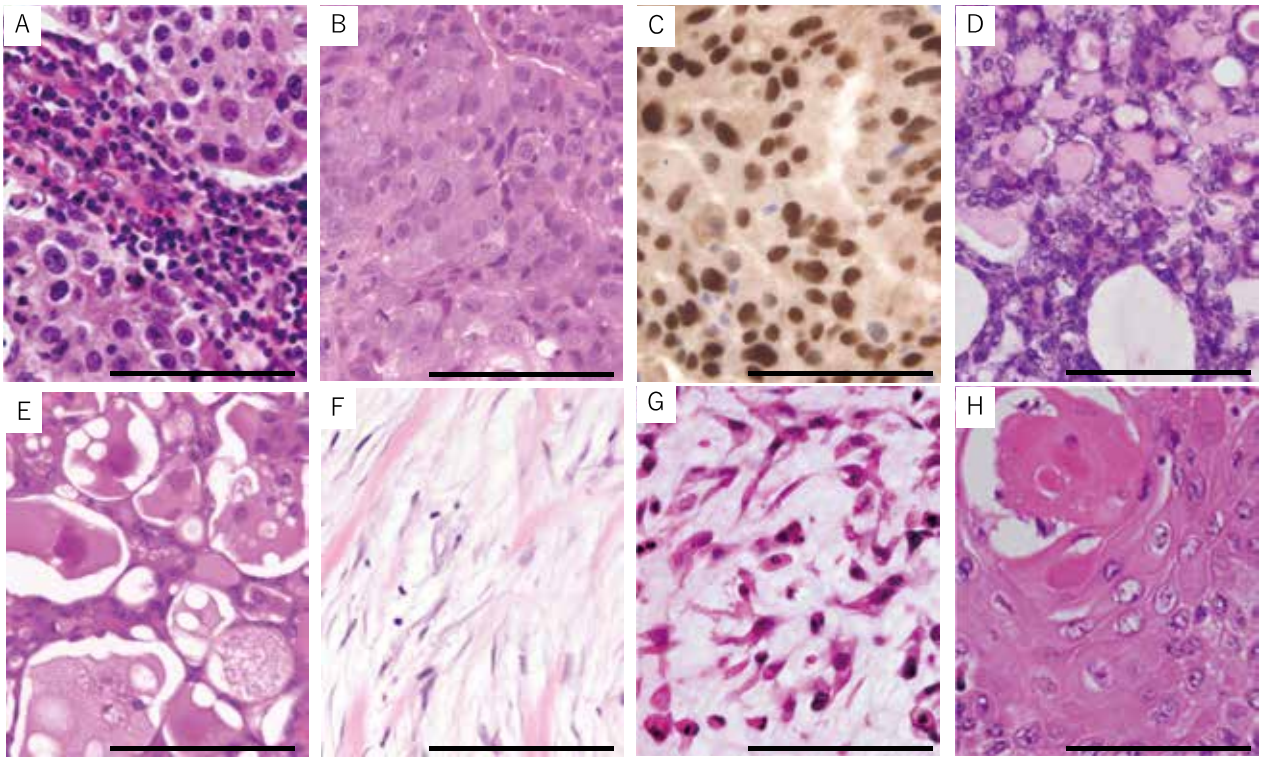

Figure 2. Representative histological appearance of rare triple-negative breast cancer (TNBC). (A) Carcinoma with medullary pattern. Carcinoma cells show high histological grade and syncytial architecture with no glandular structures. (B, C) Carcinoma with apocrine differentiation. Carcinoma cells exhibit abundant, granular eosinophilic cytoplasm and enlarged nuclei with prominent nucleoli (B). Immunohistochemistry for androgen receptor showing strong positive staining (C). (D) Adenoid cystic carcinoma. Neoplastic cells are arranged in tubular and cribriform patterns. The pseudolumina are filled with stromal matrix including basement membrane. (E) Secretory carcinoma. The tumor cells are arranged in a microcystic growth pattern with extracellular secretion, simulating thyroid follicles. (F) Fibromatosis-like metaplastic carcinoma. Spindle cells with low-grade or no atypia are arranged in wavy fascicles. (G) Matrix-producing carcinoma. The discohesive cells are contained in a chondroid matrix. (H) Metaplastic carcinoma, squamous type shows carcinoma cells with abundant eosinophilic cytoplasm and focal keratinization. Scale bars, 100 μm.

a favorable clinical course (33). Inhibitors that target *NTRK3* have recently been developed and show efficacy in patients with secretory breast carcinoma (34).

Metaplastic carcinomas are a heterogeneous group of tumors characterized by metaplastic differentiation of the neoplastic epithelium to squamous and/or mesenchymal cells. Metaplastic carcinomas show genetic similarities with the MSL subtype in Lehmann's classification and the claudin-low subtype (35). In general, metaplastic carcinoma exhibits a low survival rate and resistance to chemotherapy (36), but some cases of fibromatosis-like metaplastic carcinoma have been associated with a favorable clinical outcome (37).

Each rare TNBC type has characteristic pathological findings and a distinct clinical course. Therefore, optimal treatment for these tumors requires an accurate pathological diagnosis.

CLASSIFICATION OF TNBC AND TARGETED THERAPY

TNBC is being refined based on its molecular characteristics and clinical response to targeted therapies. These molecular subtypes show distinctive clinical behaviors, including treatment responses (Table 1). According to Lehmann *et al*., there is overlap between immune-enriched subtypes and other subtypes. Regardless of tumor subtype, the IM component for each tumor was highly correlated with percentage of tumor-infiltrating lymphocytes. The IM subtype likely reflects the presence of gene expression contributed by immune infiltrates with the carcinoma cells having the signature of a different subtype. BL1, BL2, MSL, and LAR-classified tumors had representatives with high correlations with the IM subtype. In contrast, M-classified tumors had a very low correlation with the IM group (17) (Figure 3).

Basal-like subtype

For the basal-like subtype, cell cycle and DNA damage response pathways are significantly activated, which results in increased cell proliferation. Therefore, targeting the DNA damage response pathways is a rational therapeutic approach. This subtype is highly sensitive to platinum drugs and poly (ADP-ribose) polymerase (PARP) inhibitors (38, 39). *BRCA1* and *BRCA2* function as tumor suppressor genes and play major roles in the DNA repair systems, specifically in repairing double-stranded breaks by homologous recombination. When homologous recombination is dysfunctional (homologous recombination deficiency, HRD), which is commonly observed in cases with *BRCA1/2* mutations, double-stranded breaks result in genomic instability (40). PARP1 binds to single-stranded breaks during the DNA repair process. PARP inhibitors can trap PARP1 and induce cell death by preventing this single-stranded break repair. Double-stranded breaks occur in patients with *BRCA* mutations that lack a functional homologous recombination pathway (41).

Masuda *et al*. have reported a retrospective analysis of TNBC

Table 1. Triple-negative breast cancer molecular subtype and potential targets for therapy.

Subtype	Molecular Features	Important Markers	Therapeutic Targets/Drugs
BL1	Elevated cell cycle and DNA damage response gene expression	*ATR/BRCA, Ki-67*	Cisplatin, PARP inhibitors
BL2	Enriched in growth factor signaling, metabolic pathways, and myoepithelial marker expression	*EGFR, IGF1R, NGF, MET, Wnt/b-catenin, EPHA2, TP63*	Growth factor inhibitors
IM	Genes involved in cytokine and immune signaling transduction pathways	*IL-12, IL-7, NFKB, TNF, JAK/STAT*	PD-1/PD-L1 inhibitors, other immune checkpoint inhibitors
M	Gene expression for EMT, cell motility, and differentiation	*Wnt, ALK, TGF-β*	PI3K/mTOR inhibitors, growth factor inhibitors, Src inhibitors
MSL	Increased growth factor signaling compared with (M), low proliferation, enrichment of genes associated angiogenesis and stem cells, low claudin expression	*EGFR, PDGF, ERK1/2, TGF-β, Wnt/β-catenin*	PI3K/mTOR inhibitors, growth factor inhibitors, Src inhibitors
LAR	Increased in hormonally regulated pathways, AR signaling, high rate of PIK3CA activating mutations	*AR , FOXA1, KRT18, XBP1*	AR antagonists, PI3K inhibitors, Hsp inhibitors

Modified from Lehmann *et al.* (16) ; ALK, anaplastic lymphoma kinase ; ATR, ataxia telangiectasia and Rad3- related protein ; EGFR, epidermal growth factor receptor ; EMT, epithelial-mesenchymal transition ; EPHA2, EPH receptor A2 ; ERK, mitogen-activated protein kinase ; FOXA1, forkhead box A1 : Hsp90, heat shock protein 90 ; IGF1R, insulin like growth factor 1 receptor ; IL, interleukin ; JAK, Janus kinase ; MET, hepatocyte growth factor receptor ; mTOR, mechanistic target of rapamycin ; NF-kB, nuclear factor-kappa B ; NGF, nerve growth factor ; PARP, poly(ADP-ribose) polymerase ; PD-1, programmed cell death 1 ; PDGF, platelet-derived growth factor ; PD-L1, programmed cell death 1-ligand 1 ; PI3K, phosphoinositide 3-kinase ; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha ; Src, SRC proto-oncogene ; STAT, signal transducer and activator of transcription ; TGFβ, transforming growth factor beta ; TNF, tumor necrosis factor ; Wnt, Wnt family member ; XBP1, X-box binding protein 1.

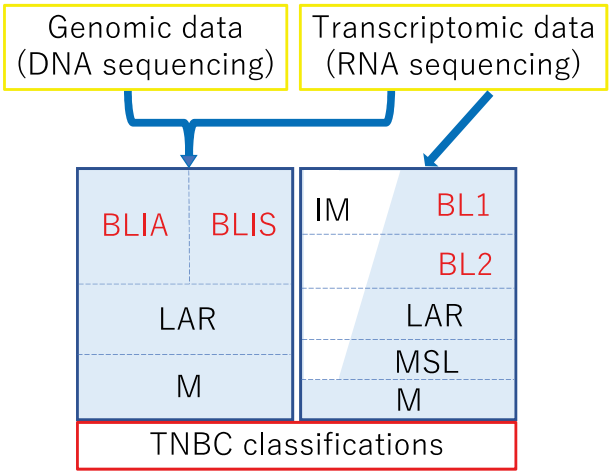

Figure 3. Classification of triple-negative breast cancer from genomic and transcriptomic data. BL1, basal-like 1 ; BL2, basal-like 2 ; BLIA, basal-like immune-activated ; BLIS, basal-like immune-suppressed ; LAR, luminal androgen receptor ; IM, immunomodulatory ; MSL, mesenchymal stem-like ; M, mesenchymal ; TNBC, triple-negative breast cancer. Modified from Lehmann *et al.* (16, 17), Burstein *et al.* (20), and Fig.2.88 in WHO classification of tumours, 5th edition, breast tumours.

subtype based on response rates to neoadjuvant chemotherapy. The BL1 subtype achieved the highest pathological complete response rate (52%), while the BL2 subtype was found to have the lowest response rate (0%) (42). Standard chemotherapeutics, palatinum salts, and PARP inhibitors may be the most effective options currently available for patients with the BL1 subtype. BL2 has unique gene ontologies involving growth factor signaling, such as the EGF, MET, and IGF1R pathways. BL2 subtype patients may be candidates for EGFR or IGF1R inhibitors.

Immune-enriched subtype

This subtype includes tumors enriched for genes associated with immune cell processes. This phenotype is common in the IM subtype as classified by Lehmann *et al.* (16) and the BLIA subtype as classified by Burstein *et al.* (20). Immunotherapies, including immune checkpoint inhibitors, have attracted attention for TNBC treatment.

Programmed death-1 (PD-1) is a T-cell inhibitory receptor that regulates the immune system by downregulating the T-cell response upon binding with its ligand, PD-L1, which is expressed in cancer cells. While activation of this pathway protects cancer cells from immune cell-mediated death, inhibition of PD-1 or PD-L1 can restore the antitumor effects of T-cells. In patients with TNBC, PD-L1 is expressed on tumor-infiltrating immune cells as well as on tumor cells. When at least 1% or more of immune cells are immunohistochemically positive for PD-L1 in tumor tissue and at the tumor margin, targeted therapy with anti-PD-L1 monoclonal antibodies may be considered (43) (Figure 4).

Mesenchymal subtype

The mesenchymal and mesenchymal stem-like subtypes are characterized by gene clusters associated with cell motility, matrix interactions, growth factors, and epithelial-mesenchymal transition. Lehmann *et al.* proposed that cell models for mesenchymal and mesenchymal stem-like TNBC may be sensitive to mTOR inhibitors because these cells exhibit activated PI3K/AKT/mTOR signaling resulting from the mutation of *PIK3CA* or deactivation of PTEN (16).

Luminal AR subtype

The AR belongs to the nuclear steroid hormone receptor family and regulates cell proliferation, apoptosis, and promotes cell migration and invasion in TNBCs (44). It is expressed in TNBCs, primarily in the LAR subtype, and is associated with apocrine histologic features (45). Barton *et al.* demonstrated that AR suppression enhances sensitivity to chemotherapy by decreasing the resistant cancer stem cell-like population (46). Lehmann *et al.*

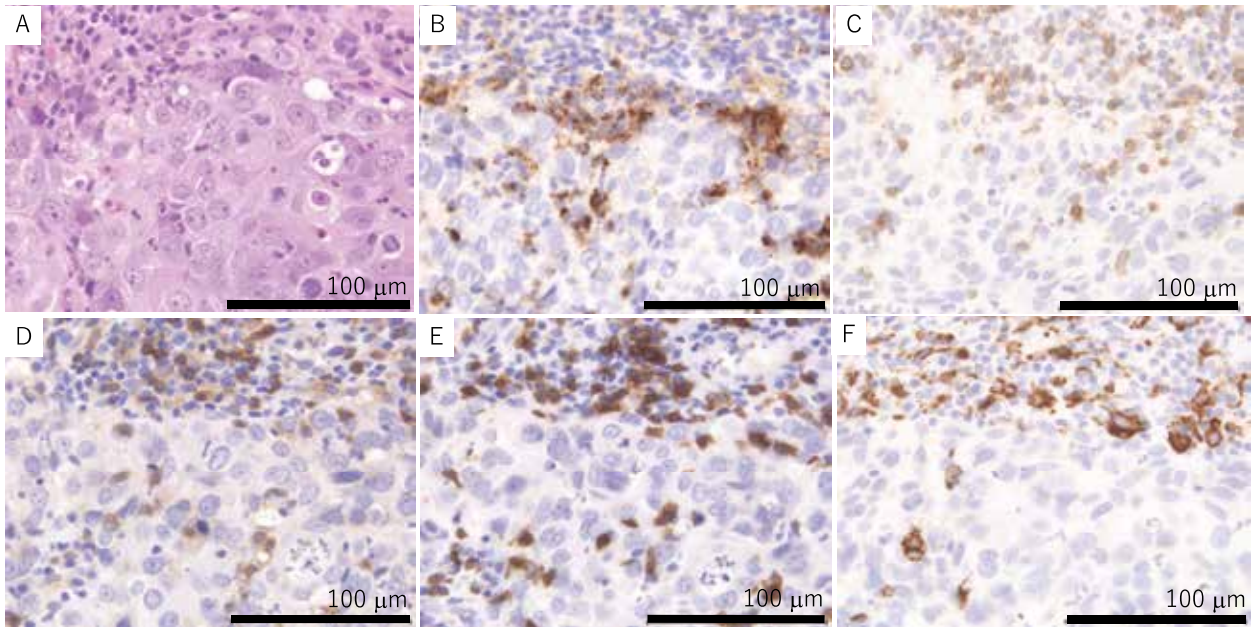

Figure 4. Histological appearance of triple-negative breast cancer (TNBC) with dense immune cell infiltration. High-grade feature with large nucleus, prominent nucleoli, and remarkable mitotic activity. H&E (A). Immune cells in the tumor tissue and at the margin of the tumor tissue exhibit positive immunohistochemical staining for PD-L1 (B) and PD-1 (C). Immune cells include CD4-positive T lymphocytes (D), CD8-positive T lymphocytes (E), and CD68-positive macrophages (F). Scale bars, 100 μm.

showed that *PIK3CA* gene mutations frequently occur in AR-positive patients with TNBC. A synergistic effect was observed with the combination of AR antagonists and PI3K inhibitors in an AR-positive cell line and xenograft models (47). AR and PI3K inhibition have been proposed as therapeutic strategies (48).

CONCLUSION AND OUTLOOK

The advent of next-generation sequencing that can be used to analyze expression changes in many genes has paved the way for genomic medicine. Nucleic acids contained in formalin-fixed paraffin-embedded tissue blocks used for routine pathological diagnosis may also be used for genomic analysis. A sufficient amount of high-quality nucleic acids is available following the preparation and examination of paraffin blocks (49). Block selection, indicating the tumor areas on the slide glass for macrodissection, and determination of tumor content have also become important pathology tasks (50). It is important for clinicians and pathologists to share data and strive for high-quality pathological and genomic diagnoses. The concept of breast cancer subtype classification is expected to change with advances and widespread use of molecular biological tests such as oncogene and multigene assays. Understanding and accepting the concept of new subtypes is important, but it is also important to closely observe the findings of histopathological specimens and provide accurate and detailed information.

CONFLICTS OF INTEREST

All authors declare no conflicts of interests.

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