Clinicopathological features and treatment strategy for triple-negative breast cancer

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Abstract

Breast cancers are divided into at least 4 subtypes based on gene expression profiles and expression of receptors (hormone receptors (HR) and HER2) as measured by immunohistochmistry. These subtypes have different prognoses and responses to treatments such as endocrine manipulation, anti-HER2 therapy and chemotherapy. Triple-negative breast cancer (TNBC) is immunohistochemically defined as lacking estrogen and progesterone receptors and not overexpressing HER2. TNBC accounts for about 15% of breast cancer patients, and is more chemosensitive but has a worse prognosis than the HR-positive/HER2-negative phenotype. TNBC is a heterogeneous disease that does not offer specific targets like HR-positive and HER2-positive breast cancers, and is similar to basal-like breast cancer and BRCA1-related breast cancer. At present, the lack of highly effective therapeutic targets for TNBC leaves standard chemotherapy, such as anthracycline and taxane combination, as the only medical treatment, but this is insufficiently efficacious. Novel approaches for TNBC such as DNA damaging agents, PARP-1 inhibitors, receptor tyrosin kinase inhibitors (TKIs) and antiangiogenesis agents are examined in clinical setting. Concerning therapeutic strategies for TNBC, it is most important to develop novel effective approaches for TNBC treatment and high-throughput predictive tools for standard chemotherapy and novel agents.

Short abstract

Triple-negative breast cancer is chemosensitive but has a poor prognosis High-throughput predictive tools for chemotherapy and novel alternatives to standard chemotherapy are urgently needed.

Key words; triple-negative breast cancer, basal-like breast cancer, PARP1 inhibitors, neoadjuvant chemotherapy, adjuvant chemotherapy

Introduction

Breast cancer is a heterogeneous disease in terms of gene expression, morphology, clinical course and response to treatment. Gene expression profiing divides breast cancer into several subtypes including luminal, HER2-enriched, basal-like (BL) and normal-like subtypes.[1] In addition, breast cancer can be divided into 4 subtypes (hormone receptor(HR)-positive/HER2-negative, HR-positive/HER2-positive, HR-negative/HER2-negative and HR-negative/HER2-positive) based on immunohistochemical analysis and treatment targets. Triple-negative breast cancer (TNBC) is defined as showing an absence of estrogen receptor (ER)-alpha and progesterone receptor (PR), and no protein overexpression or gene amplification of HER2. Many other forms of breast cancer have several effective therapeutic targets such as ER for endocrine therapy and HER2 for anti-HER2 therapy. TNBC has no effective therapeutic target at present. Consequently, only conventional chemotherapy is currently used in clinical practice and its effectiveness is limited.

In this review, we focus on clinicopathological feature, biological characteristics, effects of chemotherapy, and novel targeted therapies for TNBC.

Clinicopathological features for triple-negative breast cancer

In unselected large cohorts, the prevalence of TNBC is 11% to 17% of all breast cancer patients.[2-5] Its prevalence is higher in American-African patients (20-27%) than in white women (10-16%).[5-7] Of 11,705 in Japanese breast cancer patients, the prevalence of TNBC was 15.5%.[8] Table 1 shows the clinicopathological characteristics according to tumor subtype classified by HR and HER2 status in Japanese cases using surveillance data from the Japanese Breast Cancer Society. Among TNBC patients in this dataset the proportion of premenopausal patients is only 28%,[8] however in a population-based study from the California Cancer Registry, 37% of TNBC patients were less than 50 years old.[2] TNBC may be less common among premenopausal women in Japan than in Western countries. Several other risk factors for TNBC have been identified, including higher parity, young age at the time of first birth, use of oral contraceptives in women less than 40 years old, younger age at diagnosis, Hispanic ethnicity, lower socioeconomic status, increased body weight and metabolic syndrome.[9, 10]

Clinical characteristics of TNBC are early relapse and poor prognosis due to its aggressive phenotype, as discussed further below. In a population-based study, patients with TNBC or basal-like breast cancer (BLBC) have poorer survival than those with other breast cancers.[2, 4] Triple negativity is an independent prognostic factor regardless of tumor size,

tumor grade, nodal status and treatment.[4, 11] In patients with TNBC, risk of recurrence rises rapidly in the first 3 years, peaking 1-3 years after diagnosis, and the majority of recurrences occur within 5 years after diagnosis.[12, 13] TNBC is more likely than non-TNBC to metastasize to the brain and viscera.[14] In particular, the risk of visceral recurrence within 5 years after diagnosis is significantly higher in TNBC patients, although the risk of bone recurrence in the same interval is significantly lower.[15] Furthermore, the survival time from recurrence to death in patients with TNBC was significantly shorter than in patients with other subtypes. [12, 16, 17] There are conflicting reports concerning local failure in TNBC. Several reports showed no difference in locoregional recurrence between TNBC and non-TNBC.[18, 19] On the other hand, other groups demonstrated that TNBC was significantly associated with increased locoregional failure.[20, 21] In the largest study to date, Voduc et al showed that locoregional failure after mastectomy with or without adjuvant radiation was significantly increased in HER2-enriched subtype and basal-like (BL) subtype in TNBC (core basal-TNBC) but not non-BL in TNBC (non-core basal-TNBC). In contrast, significantly higher regional but not local failure after breast-conserving surgery plus adjuvant radiation was found in the core basal-TNBC.[22]

Histopathological features associated with TNBC are large tumor size, pushing borders, poor tubule formation, high grade with high mitotic index and marked nuclear

pleomorphism, central fibrosis and necrosis, and prominent lymphocytic infiltration.[23-26] The most common histological type for TNBC is invasive ductal carcinoma, not otherwise specified (NOS). However, TNBC also involves some special histological types including invasive lobular carcinoma (pleomorphic type), medullary carcinoma, metaplastic carcinoma, myoepithelial carcinoma, neuroendocine carcinoma, apocrine carcinoma, adenoid cystic carcinoma, and secretory breast carcinoma.[8, 23-26] Morphological classification of these special types is very important because the prognosis and response to chemotherapy is different for each type. Metaplastic carcinoma has a more aggressive phenotype and worse prognosis than other TNBC.[27] On the other hand, medullary carcinoma has a better prognosis and little benefit from aggressive chemotherapy despite high grade and basal/myoepithelial features.[28] Myoepithelial carcinoma and adenoid cystic carcinoma also have more favourable prognoses.[23, 26] Apocrine carcinoma is different from other special types in terms of prognosis. Its clinical outcome is associated with tumor grade and the disease stage.[23, 26] Recently, Honma et al reported that ER-beta1 positivity was also correlated with favorable clinical outcome in apocrine carcinoma with triple negative phenotype.[29]

The biological characteristics of TNBC have been widely examined and are summarized in Table 2. In TNBC, basal cytokeratin (CK 5/6, 14, 17) and myoepithelial markers

(smooth muscle actin (SMA), s-100, p63, CD10, vimentin, P-cadherin, osteonectin) are expressed. In addition, growth factors (epidermal growth factor (EGFR), platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1)), cell cycle regulators (Cyclin E), caveolins 1 and 2, and c-kit are more frequently overexpressed than in non-TNBC.[12, 30-37] Ki-67 labeling index is also higher in TNBC than in non-TNBC.[38]. On the other hand, decreased expression of p27 and loss of PTEN in TNBC have been reported.[32] Although EGFR overexpression and increased EGFR gene copy number occur at higher incidence in TNBC than in HR-positive and HER2-negative breast cancer, EGFR mutation was not found in TNBC.[38] Nuclear accumulation of p53 protein and TP53 mutation are more frequent in TNBC than in non-TNBC.[1, 39] However, the incidence of cyclin D1 overexpression and amplification is lower in TNBC than in non-TNBC.[39] Overexpression of topoisomerase II alpha (TOP2A) is more frequent in TNBC than in non-TNBC, but no *TOP2A* gene amplification was observed.[39] Furthermore, master cell cycle regulator *cMYC* gene amplification was rare[39] and KRAS mutation was not found in TNBC. [40]

Cytogenetic instability is also frequently observed in TNBC.[41-43] Bergamaschi et al investigated DNA copy number alterations in breast tumors using a genome-wide array-based comparative genomic hybridization survey. They demonstrated that higher numbers of

gains/losses were associated with the basal-like subtype.[41]

TNBC as determined by immunohistochemistry (IHC) has clinicopathological overlap with BLBC in intrinsic subtype determined by cDNA microarray analysis,[44] but is not identical. Cancer showing BL phenotype comprises 71-91% of TNBC, whereas about 80% of BLBC is triple negative.[45-47] Nielsen et al have developed an IHC panel for detecting BLBC with 5 markers (ER-negative, PR-negative, HER2-negative, CK5/6-positive and/or EGFR-positive) which was 76% sensitive and 100% specific. Thike et al have also shown that a tri-panel of CK14, EGFR and 34βE12 classified 84% of 653 TNBC patients as basal-like subtype, which was 78% sensitive and 100% specific.[25] Rakha et al extensively examined biological differences between BL subtype (core basal-TNBC) and non-BL in TNBC (non-core basal TNBC) using tissue microarray (TMA) (Table 3). The expression of ER-beta1, luminal-associated cytokeratin CK19, P-cadherin, MUC2, neuroendocrine differentiation markers (chromogranin A and synaptophysin), p53, tumor suppressor FHIT, cell cycle regulators (p16, cMYC and phospho-histone 3), hypoxia-related factor CA9, immunity-related markers (melanoma antigen family A-1 (MAGE1) and HLA class I heavy chain, which binds to cytoplasmic domain of HLA-B and HLA-C (HC10)) were more frequently found in the core basal-TNBC than the non-core basal-TNBC.[48] BRCA 1 germ-line mutation was also

significantly higher in the core basal-TNBC.[48] Furthermore, the core basal-TNBC has significantly poorer prognosis than the non-core basal-TNBC, even among patients treated with adjuvant anthracycline-containing regimen.[48, 49] [3] These results suggest that TNBC is a markedly heterogeneous disease, with the core basal-TNBC being quite different from the non-core basal-TNBC. In addition, Kreike et al have shown that BLBC is itself heterogeneous and can be further divided into at least 5 distinct subgroups based on gene-expression profiling. [34]

TNBC, BLBC and BRCA1-related breast cancer

BRCA1 plays an essential role in repair of double-stranded DNA breaks via homologus recombination.[50] Therefore, loss of BRCA1 function leads to genomic instability and increases the risk of developing malignant disease. Women with germ-line mutations in BRCA1 are predisposed to breast, ovarian and other malignancies.[51] Morphological, phenotypic and molecular features of BRCA1-related cancer resemble those of TNBC and BLBC. BRCA1-related cancer has pushing borders and prominent lymphocyte infiltration as morphological features.[52] BRCA1-related cancer also is characterized by ER negativity, HER2 negativity, high nuclear grade, high ki67 labeling index, and basal marker expression

(CK5/6, 14, 17, EGFR)[50, 53] Furthermore, BRCA1-related cancer shows TP53 mutation,[54] X-chromosome inactivation[55] and cytogenetic instability.[56] BRCA1 germ-line mutation is rare in sporadic breast cancers.[57] However, TNBC/BLBC share pathological and biological characteristics with BRCA1-related cancer, as described above. BRCA1 mRNA was reduced in TNBC/BLBC compared with control.[38] Non-genomic BRCA1 dysfunction may be induced in TNBC by mechanisms such as BRCA1 promoter methylation[57] and overexpression of ID4, which is negative regulator of BRCA1.[58] In the case of tumors with BRCA1 dysfunction, therapy targeting the impaired double strand DNA repair is promising, as described further below.

Treatment strategy for TNBC

Efficacy of conventional chemotherapy for TNBC

Neoadjuvant setting

Table 4 shows in detail the pathological complete response (pCR) rates for TNBC/BLBC and non-TNBC/non-BLBC treated with neoadjuvant chemotherapy. Most reports found that Anthracycline-based therapy produced higher pCR rates in TNBC/BLBC patients than non-TNBC/non-BLBC.[59-61] Furthermore, the pCR rates were improved by addition of

taxane to anthracycline.[59, 61] Thus, TNBC is more sensitive to chemotherapy than non-TNBC. However, it has worse a prognosis for overall survival (OS) and disease-free survival (DFS) than non-TNBC. This phenomenon is called the “triple negative paradox”.[60] Patients with TNBC who did not achive pCR had significantly decreased OS compared to those with non-TNBC who did not achieve pCR (HR=1.5; 95%CI, 1.3 to 1.8; P<0.0001). On the other hand, there was no significant difference in OS between TNBC patients who achieve pCR and non-TNBC patients who achieve pCR (HR=1.7; 95%CI, 0.7 to 4.2; p=0.24).[30] This paradox may be caused by the small difference in pCR rate between TNBC (around 30%) and non-TNBC (around 10%) treated with sequential therapy of standard anthracycline and taxane, and the extremely poor prognosis of TNBC patients compared to non-TNBC patients when pCR is not achieved. A treatment strategy for TNBC patients who do not achieve pCR with neoadjuvant standard chemotherapy is urgently needed.

Adjuvant setting

Chemotherapy in an adjuvant setting is a matter of debate regarding the optimal regimen and treatment schedule. In a meta-analysis of phase III trials evaluating the predictive value of HER2 and topoisomerase II alpha in early breast cancer patients treated with CMF or

anthracycline-based adjuvant therapy, the latter treatment was beneficial in patients positive for HER2 and TOP2A. In addition, among patients with TNBC, anthracycline-based therapy was superior to CMF in terms of disease-free survival with borderline statistical significance (HR=0.77, 95%CI, 0.54 to 1.09, P=0.011).[62]

A large retrospective study showed that the BL subtype of TNBC had significantly worse outcome than non-BL TNBC, and among TNBC patients treated with anthracycline-based adjuvant chemotherapy, the BL subtype had significantly lower 10-year breast cancer specific survival (BCSS) than equivalently treated non-BL subtype (HR=4.26, 95%CI, 2.00 to 9.08; $p=7.41 \times 10^{-5}$). In contrast, among TNBC patients treated with non-anthracycline-based chemotherapy, outcomes were relatively poor, without significant difference in survival between the core basal and non-core basal subtypes[3] Furthermore, the efficacy of CMF and CEF in premenopausal and node positive patients was retrospectively analyzed according to tumor subtype with archival materials from the NCIC-CTCG MA5 trial. This study showed that the core basal-TNBC has poorer 5-year OS than all other subtypes in patients treated with CEF (HR=1.84, 95%CI, 1.09 to 3.11, P=0.02), but no such difference in 5-year OS between subtypes was observed in patients treated with CMF (HR=0.90, 95%CI, 0.50 to 1.60, P=0.72).[63] Taken together, these results show only anthracycline-based

treatment might be less effective than CMF in adjuvant chemotherapy for patients with BLBC.

Combined anthracycline and taxane is one of the most beneficial chemotherapies for breast cancer, but the benefit of this combination therapy in TNBC is inconclusive. The CALGB 9344 trial evaluated addition of paclitaxel to the anthracycline doxorubicin plus cyclophosphamide (AC) in patients with node-positive early breast cancer. Addition of this taxane improved DFS and OS over AC alone in this study population. Retrospective evaluation according to tumor subtype showed that addition of paclitaxel was beneficial in TNBC and HER2-positive patients but not in ER-positive/HER2-negative patients.[64]

The BCIRG trial compared docetaxel, doxorubicin and cyclophosphamide (TAC) with fluorouracil, doxorubicin and cyclophosphamide (FAC) in patients with operable node-positive breast cancer. Prospective randomized comparison showed TAC was superior to FAC in terms of DFS and OS in the whole trial population. Retrospective analysis using 91% of this population showed that there was marginal significance for TAC vs FAC in TNBC (3-year DFS, 73.5% in TAC vs. 60.0% in FAC, HR=0.50, 95%CI, 0.29 to 1.00, *P*=0.051) as well as the HER2-positive subgroup.[11] The TACT trial, which compared FEC for 4 cycles followed by docetaxel for 4 cycles with control chemotherapy (FEC for 8 cycles or epirubicin for 4 cycles followed by CMF for 4 cycles) in patients with node-positive and operable breast cancer, did not

show an overall gain from the addition of docetaxel to anthracycline chemotherapy. Subanalysis according to tumor subtype did not demonstrate that addition of taxane to standard anthracycline produced any significant difference in outcome in node-positive TNBC patients, nor in ER-positive/HER2-negative patients.[65] A meta-analysis of 4 trials of taxane-based versus anthracycline-based adjuvant chemotherapy that reported subgroup analysis according to ER/HER2 status showed that taxane-based regimens extended DFS more than anthracycline-based regimens in ER-negative/HER2-negative patients (HR=0.77, 95%CI 0.66 to 0.90) and HER2 positive patients but not in ER-positive/HER2-negative patients.[65] These findings suggest that addition of taxane to anthracycline is beneficial in TNBC patients although reported results were retrospective or exploratory subset analyses of prospective randomized studies.

Novel treatment approaches for TNBC (Figure 1)

Treatment for breast cancer with BRCA1 dysfunction

Platinum agents

Tumor cells with BRCA1 mutation or dysfunction cannot repair DNA double strand breaks via homologous recombination, resulting in cell death. These cells have increased

sensitivity to DNA-damaging agents such as platinum and topoisomerase I inhibitors.[66, 67] In 28 TNBC patients treated with neoadjuvant cisplatin for 4 cycles, the pCR rate was 22%. Exploratory analysis for prediction of cisplatin response showed that young age (P=0.001), low BRCA1 mRNA expression (P=0.03), BRCA1 promoter methylation (P=0.04), p53 nonsense or frameshift mutations (P=0.01), and a gene expression signature of E2F3 activation (P=0.03) were significantly associated with good response.[68] Byrski et al reported interesting data for pCR rate in 102 BRCA1 mutation carriers treated with a variety of neoadjuvant chemotherapies. Of 12 patients treated with four cycles of cisplatin, 10 (83%) achieved pCR, whereas less than 25% of patients receiving conventional chemotherapy did so.[69] These data suggest platinum agents are highly effective in patients with BRCA1 dysfunction. A single-institution retrospective study of 36 metastatic breast cancer patients receiving cisplatin and gemcitabine combination therapy found a similar trend; the median progression free-survival (PFS) for TNBC patients was longer than for non-TNBC patients (TNBC, 5.3 months; non-TNBC 1.7 months, P=0.058).[70] Thus, platinum agents are promising for TNBC. However, relevant clinical evidence is limited at present. Several phase III trials to compare platinum-based chemotherapy with standard treatment are ongoing, such as the TNT trial, which is a randomised comparison of carboplatin with docetaxel for patients with advanced TNBC.[71]

PARP-1 inhibitors

Poly (adenosine diphosphate ribose) polymerase (PARP)-1, a member of the PARP family, is an essential enzyme for repair of DNA single strand breaks through base excision repair. PARP-1 binds the site of DNA damage, and then, recruits and activates repair enzymes.[72] DNA single strand breaks induced by antitumor drugs cannot be repaired by base excision repair if PARP-1 function is inhibited. Inhibition of PARP-1 subsequently leads to accumulation of DNA single strand breaks. During the S-phase of the cell cycle, the replication fork is arrested at the site of a DNA single strand break, which then degenerates into a DNA double strand break. In cells with normal BRCA function, this triggers activation of the homologus recombination pathway to repair the break[73]. In tumor cells with BRCA dysfunction, PARP-1 inhibition induces cell death because unrepaired single-strand breaks induce accumulation of double-strand breaks that are not repaired by homologous recombination. Preclinical data indicate that PARP-1 inhibitors have effective antitumor activity in the presence of BRCA dysfunction. Olaparib 800mg/day monotherapy showed 41% objective response (OR) and 5.1 months PFS in a phase II study that enrolled pretreated metastatic breast cancer patients with BRCA1/2 germ-line mutations. This treatment was feasible, with controllable

adverse events including fatigue, nausea/vomiting and diarrhea.[74] In a randomized phase II trial for TNBC, gemcitabine/carboplatin plus BSI-201 was compared with chemotherapy alone in patients within 2 prior treatments for metastatic disease. Addition of BSI-201 to chemotherapy significantly improved the objective response rate (ORR) (chemotherapy alone, 16%; BSI-201 combination, 48%; P=0.002), clinical benefit rates (CBR) (21% vs. 62%; P=0.0002), PFS (3.3 months vs. 6.9 months; P<0.0001) and OS (5.7 months vs. 9.2 months; P=0.0005). No difference in adverse events between the two groups was found.[75] PARP-1 inhibitors are among the most promising drugs for TNBC, especially with BRCA dysfunction. A phase III trial comparing gemcitaine/carboplatin with or without BSI-201 in a metastatic setting is in progress.[76]

Trabectedin

Trabectedin (ecteinascidin-743, ET-743, Yondelis), a tetrahydroisoquinoline alkaloid isolated from the Carribean tunicate *Ecteinascidia turbinata* , interacts with DNA.[77] This compound showed antitumor activity against TNBC with normal nucleotide excision repair and dysfunctional homologus recombination.[78] Trabectedin may be more effective for TNBC with BRCA dysfunction.

Molecular-targeted therapy

EGFR inhibitors

EGFR, a highly expressed protein in TNBC, may be a therapeutic target for TNBC. The TBCRC 001 trial, a randomized phase II study for stage IV TNBC, showed that the ORR and CBR for the anti-EGFR antibody cetuximab alone were only 6% and 10%, respectively, and for cetuximab plus carboplatin, 17% and 31%, respectively. The change in EGFR signaling was examined in this study, using tumor samples collected before and 1 week after initiation of treatment. Twelve of 16 samples showed EGFR expression and activated EGFR signaling before cetuximab administration. Cetuximab treatment suppressed EGFR signaling in only 4 of these 12 patients and those 4 patients responded to the treatement. Conversely, in the other 8 patients with pretreatment EGFR expression and activated signaling, the signaling in tumor tissues was persistently activated despite cetuximab therapy and the patients did not respond.[79] Constitutive activation of MAPK signaling is also involved in the de novo and acquired resistance of breast cancer cells to EGFR TKIs.[80, 81] In addition, EGFR gene amplification was found in about 20% of TNBC, but EGFR mutation was not observed. [38] EGFR inhibitors for TNBC are limited. However, they may induce increase the sensitivity to

chemotherapy, and combination therapy including EGFR-targeted agents may be more effective for TNBC.

Antiangiogenesis

Angiogenesis is essential for tumor growth, survival, progression and metastasis. Tumor angiogenesis is more active in BLBC, which has higher expression of hypoxia-related protein than non-BLBC.[48] In addition, a retrospective analysis of 679 consecutive primary breast cancers showed that TNBC has significantly higher levels of vascular endothelial growth factor (VEGF) than non-TNBC.[82] Another retrospective study using tissue micorarray analysis of archival materials from a controlled randomized trial to examine the effect of adjuvant treatment showed that VEGF receptor (VEGFR)-2 is more highly expressed in TNBC than in non-TNBC; furthermore, VEGFR-2 expression was significantly associated with decreased BCSS in TNBC.[83] Therefore, angiogenesis may be a treatment target for TNBC. In a neoadjuvant phase II trial, cisplatin and the anti-VEGF antibody bevacizumab in combination showed 78% ORR and 17% pCR.[84] Sunitinib, an orally-available multi-TKI, inhibits a wide variety of TK including VEGF-R1,2,3, platelet-derived growth factor receptor-alpha and beta, kit, glial cell-derived growth factor, and Fms-like tyrosine kinase-3-internal tandem duplication.

Sunitinib inhibits not only angiogenesis but also tumor growth. In metastatic breast cancer patients previously treated with anthracycline and taxane, sunitinib monotherapy produced ORR in 3 of 20 patients (15%).[85] Antiangiogenesis is thus another promising treatment. To confirm the value of this strategy, several phase III trials are ongoing such as the CALGB 40603 trial[86] in a neoadjuvant setting and the BEATRICE trial in an adjuvant setting.[87]

Dasatinib

Dasatinib is an oral TKI, which inhibits the TK activity of abl, the SRC family and c-kit. It suppresses growing of cells with BLBCL in vitro.[88] A phase II study of dasatinib in locally advanced breast cancer patients who were pretreated with anthracycline and taxane showed 4.7% ORR, 9.3% CBR and 8.3 weeks of PFS.[89]

Other promising agents for TNBC

Many kinds of novel approaches for TNBC are being developed in preclinical and early clinical phases. These new agents include inhibitors of mamarian target of rapamycin (mTOR, a key molecule in the phosphoinositide-3-kinase (PI3K)/Akt pathway) such as RAD001, apoptosis-inducing agents including tumor necrosis factor-related apoptosis-inducing ligand

(TRAIL) agonists such as lexatumumab, inhibitors of cell cycle regulator kinases, e.g. the check point kinase (Chk)1 inhibitor UCN-01, and epigenetic modifiers such as vorinostat that act via inhibition of histone deacetylase .[32]

Conclusions

TNBC is quite different from non-TNBC in clinical, pathological and phenotypic features. TNBC is a heterogeneous disease with a wide variety of histological subtypes although the majority of cases are classified as invasive ductal carcinoma, NOS. TNBC is not identical to BLBC, however, they overlap substantially. TNBC/BLBC is also associated with BRCA dysfunction. TNBC has relatively high chemosensitivity but poor prognosis. In the adjuvant setting, patients with TNBC are treated only with standard chemotherapy used for non-TNBC as well, because of the lack of effective therapeutic targets at present.

Concerning treatment strategy, classification of TNBC is very important. At least three systems are available such as classifications according to histological type, pathological response to neoadjuvant chemotherapy, and BL features. Classification in special histological subtypes is useful to determine whether a patient needs to receive chemotherapy. Medullary carcinoma, myoepithelial carcinoma and adenoid cystic carcinoma have better prognoses and

little benefit from aggressive chemotherapy. Pathological response to neoadjuvant chemotherapy is very important because the prognosis is good for patients who achieve pCR but extremely poor for those who do not. High-throughput predictive tools for chemotherapy and novel alternatives to standard chemotherapy are urgently needed. Patients who will not reach pCR after 6 month standard chemotherapy cannot wait until treatment termination.

Treatment for breast cancer with BRCA1 dysfunction such as PARP1 inhibitors and platinum-based chemotherapy are the most promising in novel approaches for TNBC. These treatments are more in the presence of BRCA1 dysfunction, which is associated with TNBC. However, exploiting this feature of TNBC presents some problems. There are no widely available and validated assays for BRCA dysfunction without germ-line mutation. Moreover, the relationship between the efficacy of these agents and levels of BRCA1 dysfunction without germ-line mutation is not known. These problems need to be resolved as soon as possible.

Regarding therapeutic strategies for TNBC, it is most important to develop new treatment approaches and high-throughput predictive tools for chemotherapy and novel targeted therapy.

Acknowledgments

We received from the Ministry of Education, Culture, Sports, Science and Technology of Japan grant-in-aid for scientific research #21591671 and Japan Society for the Promotion of Science (Y. Yamamoto; Researcher Number 20398217)

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Table 1. Clinicopathological feature according to tumor subtype in Japanese breast cancer patients

	Receptor subtype			
	HR+/HER2- n=8,039 (68.7%)	HR+/HER2+ n=892 (7.6%)	HER2 n=977 (8.3%)	TN n=1,797 (15.4%)
Age, median (range)	56 (NR-100)	54 (23-93)	56 (22-95)	57.5（NR-94）
Premenopausal patients	37.1%	38.8%	24.1%	28.1%
Ratio of bilateral BC	6.6%	5.9%	4.8%	6.2%
Incidence of BC family history	8.6%	8.4%	24.1%	28.1%
BMI	154.3 ± 6.3 Kg/m^2	154.9 ± 6.1 Kg/m^2	154.0 ± 6.2 Kg/m^2	153.8 ± 6.3 Kg/m^2
Tumor size	2.6 ± 2.1 cm	3.2 ± 2.2 cm	3.5 ± 2.6 cm	3.4 ± 2.7 cm
Incidence of positive node	20.6%	34.9%	38.5%	32.2%
Pathological findings				
DCIS	6.8%	6.5%	8.8%	4.8%
Papillotubular ca.	27.5%	30.0%	33.1%	23.8%
Solid-tubular ca.	14.4%	18.4%	22.8%	29.6%
Scirrhous ca.	38.7%	35.5%	22.4%	23.0%
Mucinous ca.	4.4%	2.1%	0.8%	0.5%
Invasive lobular ca.	3.9%	1.8%	0.6%	2.7%
Tubular & Secretory ca.	0.4%	0%	0%	0.2%
Medullary ca.	0.3%	0.2%	0.8%	3.4%
Metaplastic ca.	0.1%	0.3%	0.3%	2.0%
Apocrine ca.	0.2%	0%	2.1%	4.3%

BC, breast cancer; BMI, body mass index; DCIS, ductal carcinoma in situ; ca, carcinoma; HR, hormone receptor; TN, triple negative, Data from [8]

Table 2. Clinicopathological and biological characteristics of triple-negative/ basal-like breast cancer

	TN/BL BC	Non-TN/Non-BL BC	p value	Ref
Age (mean)	53	57.7	<0.0001	[12]
Tumor size (mean)	3.0 cm	2.1 cm	<0.0001	[12]
LN metastasis, positive	54%	46%	0.02	[12]
Histological grade III	66%	28%	<0.0001	[12]
LVI, present	40%	32%	0.06	[12]
EGFR, positive	44%	8%	<0.0001	[47]
EGFR mutation	0%	NA	NA	[38]
EGFR gene copy number >3	21%	2% (luminal A)	0.016	[38]
CK14, positive	52%	3%	<0.0001	[39]
CK5/6, positive	52%	5%	<0.0001	[39]
Smooth muscle actin	22%	0%	0.02	[90]
Vimentin	94%	7%	0.0001	[90]
p53 protein, positive	71%	14%	<0.0001	[39]
P53, mutation	82%	13% (luminal A)	<0.0001	[44]
p27 protein, positive	30%	44%	0.051	[53]
Ki67 labeling index, >30%	68%	6%	<0.0001	[39]
TOP2A protein, positive	79%	52%	0.008	[39]
TOP2A CISH, amplification	0%	10%	0.0859	[39]
Cyclin D1 protein, high	23%	75%	<0.0001	[39]
CCND1 CISH, amplification	0%	17%	0.029	[39]
Cyclin E protein, positive	46%	15%	0.0001	[53]
MYC CISH, amplification	4%	10%	n.s.	[39]
Caveolin 1, positive	62%	2%	<0.0001	[39]
Caveloin 2, positive	31%	2%	<0.0001	[39]
c-KIT protein, positive	31%	11%	<0.0001	[47]
VEGF levels (median)	8.2 pg/μg DNA	2.7 pg/μg DNA	<0.001	[82]
VEGFR-2, high	22%	13%	0.03	[83]
BRCA1 germ-line mutation	21%	5%	0.0001	[53]
BRCA1 mRNA	reduced		0.0001	[38]

BC, breast cancer; BL, basal-like; LN, lymphnode; LVI, lymphvascular invasion; NA, not

applicable; n.s., not significant; TN, triple-negative; Ref; References

Table 3. Biological characteristics between basal-like (core basal) and non-basal like (non-core basal) subtypes of triple-negative breast cancer (48)

	core basal (positive rate)	Non-core basal (positive rate)	P value
ER-β1	70%	56%	0.008
CK18	37	51	0.1
CK19	52%	36%	0.005
Smooth muscle actin	33%	25%	n.d.
Caveolin 1	36%	21%	n.d.
Caveolin 2	20%	20%	n.d.
E-cadherin	37%	39%	n.d.
P-cadherin	79%	61%	0.02
MUC2	10%	0%	0.03
Chromogranin A	20%	5%	0.009
Synaptophysin	7%	2%	0.004
P53	62%	41%	0.006
FHIT	70%	50%	0.004
P16	82%	63%	0.012
c-Myc	28%	5%	0.006
Phospho-histone 3	85%	0%	0.001
CA9	76%	45%	0.006
MAGE1	83%	71%	0.006
HC10	31%	21%	0.004
BRCA1 germ-line mutation	37%	4.3%	0.003

BLBC, basal-like breast cancer; HC10, HLA class I heavy chain, which binds to cytoplasmic domain of HLA-B and HLA-C; MAGE1, melanoma antigen family A-1; TNBC, triple-negative breast cancer; Ref; References, Data from [48]

Table 4. Pathological complete response to neoadjuvant chemotherapy in triple-negative/ basal-like breast cancer.

Tumor characteristics	Regimen	No.of patients	pCR rate		Ref
			TN/BL	Non-TN/non-BL	
TN	FEC100	40	13%	N. E.	[91]
	Intensified FAC	56	47%	N.E..	
	AD	145	17%	3%	[92]
	AC	107	27%	11%	[60]
	FAC/FEC/AC	308	20%	5%	[61]
	T-FAC/T-FEC	588	28%	17%	
	Single T	58	12%	2%	
	others	164	14%	7%	
	FAC/FEC	293	17%	4%	[59]
	FEC-D	187	35%	23%	[93]
	ddAD or AC-D	116	24%	6%	[94]
	AC,ddAC, AD, DC	126	34%	3% (ER+/HER2-)	[95]
	Infusional ECisF	94	17%	9%	[96]
	Cisplatin	28	22%		[68]
BL	P-FAC	82	45%	11%	[97]
	DAC	50	10%	18%	[98]
	ddAC or DCape	186	47%	21%	[99]
BRCA1/2	ACR	38	44%	4%	[100]
	CMF	14	7%		[69]
	AC	23	22%		
	FAC	28	21%		
	AT	25	8%		
	Cisplatin	12	83%		

pCR, pathological complete response; No, number; TN, triple-negaitve; BL, basal-like; Ref, reference; FEC, fluorouracil + epirubicin + cyclophosphamide; AD, doxorubicin + docetaxel; AC, doxorubicin + cyclophosphamide; FAC, fluorouracil + doxorubicin + cyclophosphamide; T-FAC, paclitaxel - FAC; T-FEC, paclitaxel - FEC; T, taxane; FEC-D, FEC-docetaxel; ddAD, dose-dense doxorubicin + docetaxel; AC-D, AC-docetaxel; DC, docetaxel + cyclophosphamide; EcisF, epirubicin + cisplatin + fluorouracil; P-FAC, paclitaxel-FAC; DAC, docetaxel + doxorubicin + cyclophosphamide; Dcape, docetaxel + capecitabine; ACR, anthracycline-containing regimen; CMF, cyclophosphamide + methotrexate + fluorouracil; AT, doxorubicin + paclitaxel; N.E., not examined.

Figure legends

Figure 1. Novel therapeutic targets and agents for their targets in TNBC