Chapter 11
Triple-Negative Breast Cancer: Subtypes with Clinical Implications

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Background

Triple-negative breast cancers (TNBC) are a heterogeneous group of malignant breast tumors traditionally defined by their lack of expression of estrogen receptor (ER), progesterone receptor (PR), and over-expression of human epidermal growth factor receptor 2 (HER-2). TNBC accounts for about 10–15 % of all breast cancers. Population-based studies show that women with high body mass index and those who reported no recreational physical activity are at a higher risk for developing TNBC than women who are physically active and those with low body mass index [1, 2]. Interestingly, some factors that are known to decrease the risk of breast cancer in general, do increase the risk of TNBC. These include first childbirth at an early age and multiparity. Racial disparity is also well-documented with African-American women having the highest incidence rates for TNBC, followed by Hispanic women [3]. The negativity of these tumors for ER and PR as well as their lack of HER-2 over-expression, render them resistant to hormonal and trastuzumab (Herceptin) therapy, making treatment a challenging task. Although, by DNA microarray analysis, most TNBC will fall into the basal-like category of breast cancers, and therefore will theoretically have a poor prognosis compared to other subtypes, basal-like breast cancer is one of several "faces" of TNBC, albeit the "ugly face." In this chapter, we will discuss the different subtypes of TNBC, their morphological features, immunophenotype, molecular background and the clinical implications of these subtypes. The immunohistochemical and molecular characteristics are summarized in Table 11.1.

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Table 11.1 Subtypes of TNBC with their characteristic immunohistochemical and molecular features

TNBC Subtype	Immunohistochemistry	Molecular characteristics
Adenoid cystic carcinoma	ER−/PR−/HER2− in >90 % C-KIT+, P63+, EGFR+HCM−, calponin—Ki67 low, TP53—low Topo IIα expression	t(6, 9)(q22–23;p23–24) MYB-NFIB fusion gene
		No EGFR gene amplification
		No KIT mutation
Metaplastic	ER−/PR−/HER2− in >90 %	Claudin-low associated
Carcinoma	Cytokeratin panel (CK903, AE1/AE3, CK903) variable P63+ (>90 %)	Low expression of GATA3-regulated genes and genes responsible for cell-cell adhesion
		Increase in markers linked to stem cell function
Carcinoma with apocrine	ER−/PR− usually HER2− (>50 %)	Gains in 1p, 1q and 2q
		Losses of 1p, 12q, 16q, 17q, and 22q19
Differentiation	GCDFP15+ (diffuse)	
	AR+, BCL2−	
Pleomorphic	ER−/PR− (all cases)	Aneuploidy and high S-phase
Carcinoma	HER2− (usually)	
	Pankeratin+, CAM 5.2+	
	EMA+ (weak and focal)	
	P53+ (71 %), Ki67 (high)	
	BCL2−	
Secretory carcinoma	ER−/PR−/HER2−. EMA+, S-100 protein+, E-cadherin+. CK5/6 and 14+	t(12, 15) ETV6-NTRK3 gene fusion. Alteration of the ETV6 gene in both the in situ and invasive components
Carcinoma with medullary features	ER−/PR−/HER2− in >90 %	EGFR gene amplification
	CK5/6, EGFR, TP53+ (variable)	TP53 gene mutation
	KI-67 high	BRCA1 gene mutations common
		Epstein-Barr virus infection?
Basal-like breast	ER−/PR−/HER2− (all cases)	TP53 mutation (83 %)
Carcinoma	CK5/6+, EGFR+ (45–70 %)	Alteration in BRCA-1 activity and loss of function
	CK14+, IMP3+, CKIT+ (45 %)	
	P53+. Others: VEGF+, maspin+	X-chromosome abnormalities
	osteopontin, Integrin β4	ID4 and cyclin E1 expression
	Caveolin1 and 2+	VEGF and Fascin expression

(+): Positive, (−): negative

Adenoid Cystic Carcinoma

Adenoid cystic carcinoma (ACC) of the breast is a rare and morphologically distinct form of breast cancer, comprising less than 1 % of all cases [4]. In contrast to other TNBC, the incidence of mammary ACC among Blacks is significantly lower than in Whites [5]. Histologically, these tumors are identical to their salivary gland counterparts. The tumor is composed of two cell types: cuboidal epithelial cells with rather abundant cytoplasm and pale nuclei lining tubular duct-like structures that contain neutral polysaccharides (PAS positive, diastase sensitive), and myoepithelial-like cells that elaborate acid mucopolysaccharides (alcian blue positive) and abundant basal lamina material. Mammary ACC can assume several architectural patterns including: solid, cribriform, tubular, and trabecular configurations. These patterns may not be distributed homogenously in a given tumor causing a potential diagnostic dilemma, especially on core needle biopsies. A predominant cribriform pattern may be confused with invasive or in situ cribriform carcinoma, and collagenous spherulosis (Fig. 11.1a). DCIS in association with ACC is seen in a minority of cases and may be difficult to distinguish from the surrounding nests of invasive carcinoma.

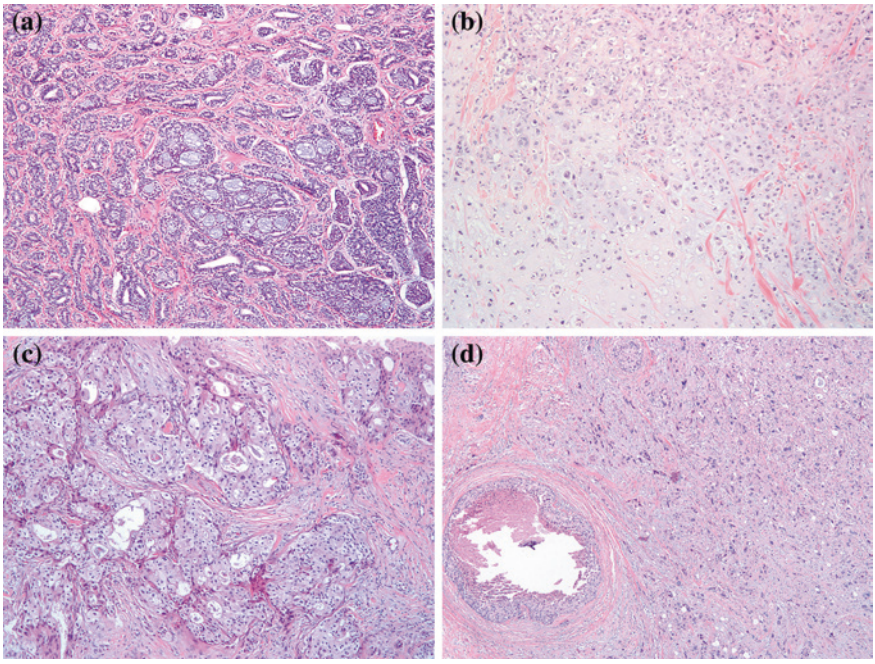

Fig. 11.1 **a** Adenoid cystic carcinoma, H&E 100×. **b** Metaplastic Carcinoma with mesenchymal differentiation, 100×. **c** Carcinoma with apocrine differentiation, 100×. **d** Pleomorphic carcinoma, 100×

Immunohistochemistry

Immunohistochemistry is helpful in cases of ACC, not only to confirm the diagnosis, but also to differentiate it from its mimickers. A panel including C-KIT (CD117), P63, heavy chain myosin, and calponin is very helpful [6]. ACC is usually positive for C-KIT and P63, and negative for both heavy chain myosin and calponin. Collagenous spherulosis and cribriform DCIS are positive for all myoepithelial cell markers but negative for C-KIT, while invasive cribriform carcinoma will not express any of those markers. Although, typically negative for ER, PR, and HER-2, up to 12 % of mammary ACC have been reported to be ER+/PR+ [5]. Other immunohistochemical studies dedicated solely to mammary ACC are identified in the literature, but are all limited by the small number of cases studied. These studies show a low proliferation index manifested by Ki-67, lack of P53 expression [7], and low Topoisomerase IIα expression, while demonstrating an over-expression of EGFR in 65 % of ACC cases [8].

Molecular Characteristics

Microarray-based gene expression studies have included ACC in the basal-like category due to their triple negative phenotype and expression of basal cell markers. However, studies focusing only on ACC show some molecular differences that distinguish ACC from the crowd of TNBC. ACC consistently displays t(6;9)(q22–23; pp 23–24) translocation, which generates a fusion transcript involving the MYB and NFIB genes [9]. This translocation is considered the key oncogenic mechanism in the pathogenesis of ACC. EGFR gene amplification which has been shown in some basal-like breast cancers, has not been demonstrated in ACC [8]. Similarly, C-KIT expression characteristic of ACC does not reflect an underlying KIT mutation.

Metaplastic Carcinoma

The term *metaplastic carcinoma* refers to a heterogeneous group of invasive breast carcinoma with microscopic features that diverge from glandular differentiation. These include either squamous or mesenchymal cell differentiation (e.g., spindle cell, chondroid, osseous, or myoid). Metaplastic carcinoma accounts for approximately 1 % of all invasive breast carcinomas. Clinically, patients present in a similar fashion to patients with invasive ductal carcinoma, NOS, in terms of their age at presentation and the manner in which the tumor is detected. The mammographic appearance of metaplastic carcinoma is not specific, except in tumors with osseous metaplasia, where bone-forming areas can be radiologically identified. Microscopically, metaplastic carcinoma varies in the type and extent of metaplastic change. In some tumors, the metaplastic foci may be present as isolated microscopic

foci in an otherwise typical invasive ductal carcinoma. In other cases, particularly tumors with squamous and spindle cell metaplasia, the metaplastic component can present in a pure form without any recognizable glandular component. The latter may be difficult to differentiate from a malignant phyllodes tumor or a sarcoma on needle core biopsies. The most common heterologous elements in metaplastic carcinoma are osseous and chondroid differentiation (Fig. 11.1b). In these tumors, the bone and cartilage may appear histologically benign or frankly malignant, further raising the possibility of a sarcoma. The presence of DCIS in tumors with a predominant mesenchymal component, supports the diagnosis of metaplastic carcinoma.

Immunohistochemistry

The diagnosis of metaplastic carcinoma in challenging cases lies in the identification of the epithelial origin of the tumor cells. This may require the use of a panel of low-and high-molecular weight cytokeratins since many metaplastic carcinomas show only focal positivity for CK or may even be negative to some. CK903(34betaE12) and P63 have been reported as sensitive markers for metaplastic carcinomas [10]. As other members of the TNBC family, >90 % of metaplastic carcinomas are negative for ER, PR, and HER2.

Molecular Characteristics

Metaplastic carcinoma are thought to arise from altered epithelial and/or myoepithelial cells. This theory is supported by cytogenetic and molecular studies that demonstrate the same clonality in both the glandular and non-glandular components of the tumor, indicating a common stem cell origin [11, 12]. Collectively, metaplastic carcinomas fall into the category of basal-like breast cancer, however, recent studies suggested that a subset of these tumors displays transcriptomic features consistent with cells undergoing epithelial-to-mesenchymal transition. These are referred to as Claudin-low tumors. Metaplastic carcinomas and claudin-low tumors are shown by comparative genomic hybridization to have low expression of GATA3-regulated genes and of genes responsible for cell-cell adhesion with enrichment for markers linked to stem cell function [13].

Carcinomas with Apocrine Differentiation

Focal apocrine differentiation can be seen in many types of breast carcinoma including: lobular, ductal, tubular, micropapillary, and even medullary carcinoma. However, carcinomas with extensive apocrine differentiation represent

approximately 4 % of breast cancer and are represented in this category [14]. Histologically, the majority of the tumor cells display features reminiscent of apocrine cells such as enlarged round nuclei with prominent nucleoli and abundant granular eosinophilic cytoplasm that is PAS-positive (Type A cells) (Fig. 11.1c). Some cells may have abundant foamy cytoplasm, which is referred to as Type B cells, while other tumors may have a combination of both.

Immunohistochemistry

A typical apocrine carcinoma shows diffuse positivity for GCDFP-15 [14], and BCL-2 negativity. Staining for ER, PR is usually negative. A portion of these tumors is also negative for HER2 protein over-expression (triple negative). Androgen receptor expression in ER-negative breast tumors was found to be associated with apocrine differentiation [15].

Molecular Characteristics

The immunophenotypic signature described above has inspired researchers to look for a similar "*apocrine molecular signature.*" Microarray studies show increased androgen signaling and overlap with the HER2 group of tumors. However, this proposed molecular signature does not correlate well with apocrine morphology. Approximately, only half of carcinomas with apocrine differentiation show this molecular signature. Comparative genomic hybridization has identified several copy number alterations in carcinomas with apocrine differentiation including gains of 1p, 1q and 2q, as well as losses of 1p, 12q, 16q, 17q, and 22q [16]. However, these are also common alteration regions that are seen in breast carcinoma in general. This data suggests that although carcinomas with apocrine differentiation may have a characteristic morphology and immunophenotype, they do not represent a distinct molecular entity.

Pleomorphic Carcinoma

This unusual and rare tumor is considered a variant of high-grade invasive ductal carcinoma, NOS. Morphologically, it is characterized by proliferation of pleomorphic, bizarre cells with greater than sixfold variation in nuclear size. Multinucleated tumor giant cells are common and account for more than 50 % of the tumor cells. Areas of conventional adenocarcinoma may be present. However, pure pleomorphic carcinoma and cases associated with metaplastic carcinoma, especially of the spindle cell type, may be seen and can be misdiagnosed as

sarcoma or metastatic tumors. The presence of adjacent foci of ductal carcinoma in situ supports a breast primary in challenging cases (Fig. 11.1d). Axillary lymph node metastases are present in almost half the cases.

Immunohistochemistry

In the original series by Silver and Tavassoli, all tumors showed strong, diffuse positivity for pan-cytokeratin and CAM 5.2, which is useful in differentiating these tumors from sarcomas. EMA was positive in areas of conventional ductal carcinoma, but was very weak and focal in the multinucleated tumor cells. All tumors were negative for ER and PR. HER-2 was also negative in the majority of cases, especially those with node-negative disease [17]. P53 expression was present in 71 %, but none expressed bcl-2. Ki-67 proliferation index was also increased with a mean of 33 %.

Molecular Characteristics

The data on pleomorphic carcinoma is very sparse due to the rarity of these tumors. Nevertheless, the majority of these tumors show aneuploid DNA content and high S-phase.

Secretory Carcinoma

Secretory carcinoma (SC) is an exceptionally rare, low-grade carcinoma accounting for <0.15 % of all breast cancers. They occur over a wide age range, but more commonly in children and young adults "*Juvenile carcinoma.*" Clinically, they are well-circumscribed tumors, located close to the areola. Grossly, the tumor size ranges from 0.5 to 12 cm with an average of 3 cm. Microscopically, secretory carcinoma has pushing borders and is composed of polygonal cells with granular eosinophilic to foamy cytoplasm (Fig. 11.2a). A consistent finding is the presence of intracellular and extracellular, eosinophilic, secretory material that is positive for PAS and alcian blue (Fig. 11.2b, c). The tumor displays one or more of three growth patterns: solid, tubular, and microcyctic. The latter resembles thyroid follicles. Most tumors contain a mixture of all three patterns.

Immunohistochemistry

The tumor cells are negative for ER, PR, and HER-2, and frequently positive for epithelial membrane antigen (EMA), S-100 protein, and E-cadherin (Fig. 11.2d). Expression of basal cytokeratins (CK 5/6 and 14) was also identified in five out of

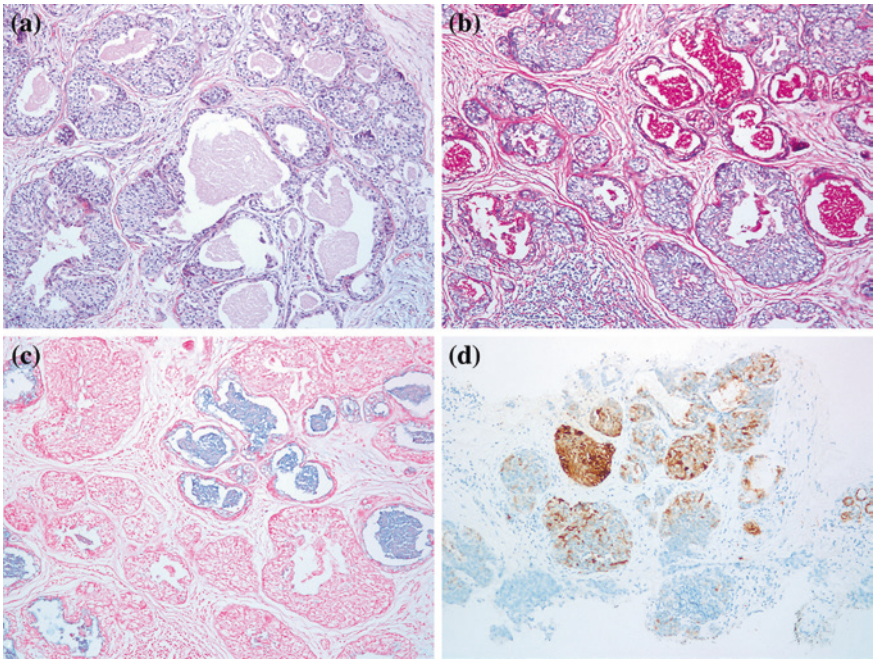

Fig. 11.2 Secretory carcinoma. **a** H&E, 100×. **b** Luminal secretions are strongly PAS-D positive. **c** Alcian blue positive. **d** S-100 protein is also strongly positive

six cases in one study, suggesting that secretory carcinoma belongs to the basal-like group of breast cancer [18].

Molecular Characteristics

In 2002, Tognon et al. [19] have shown that secretory carcinoma is characteristically associated with t(12, 15) that results in ETV6-NTRK3 gene fusion, the same translocation which was originally described in congenital fibrosarcoma and cellular mesoblastic nephroma. Additionally, FISH analysis shows alteration of the ETV6 gene in both the in situ and invasive components [18].

Carcinoma with Medullary Features

Classic medullary carcinoma (MC) is very rare, representing less than 1 % of all breast cancers. The diagnosis of classic MC requires stringent diagnostic criteria which include histological circumscription, lack of tubular formation with

syncytial architecture in >75 % of the tumor, intense lymphoplasmacytic infiltration, and highly pleomorphic tumor cells with numerous mitoses. Tumors that lack some of these features are classified as "atypical medullary carcinoma" or "invasive ductal carcinoma with medullary features." However, these criteria are often difficult to apply resulting in a high interobserver variability. For the same reasons, the new WHO Classification of Tumors of The Breast has now grouped classic and atypical medullary as well as a subset of invasive carcinoma of no special type under "Carcinomas with medullary features". Foci of squamous metaplasia can also be seen in MC and should not be considered as a metaplastic carcinoma.

Immunohistochemistry

The majority (>90 %) of MC are negative for ER, PR, and HER-2, with variable expression of basal cytokeratin (CK5/6), EGFR, and P53. The intense lymphocytic infiltrate is predominantly CD3+T lymphocytes. Not surprisingly, MC shows a high proliferation index with Ki-67.

Molecular Characteristics

MC heirs a lot of its molecular features from its basal-like family of breast cancers, including EGFR gene amplification, TP53 gene mutation, and increased incidence in patients with BRCA1 gene mutations [20]. Some questioned the role of Epstein-Barr virus infection in MC given its morphologic similarities with lymphoepithelial carcinomas of other organs. Whereas, one study showed an association between Epstein-Barr virus and MC, another study failed to reproduce this link [21, 22].

Basal-like Breast Carcinoma

Basal-like breast carcinomas (BLBC) is a distinct group of breast carcinoma that has evolved as a separate molecular subtype from gene expression profiling studies [23, 24]. They usually present as rapidly growing breast masses, most probably as "interval breast cancers" (those diagnosed between annual mammograms) [25]. Radiologically, they are often ill-defined, oval, round, or lobulated masses. Extensive necrosis may give the impression of a partially solid and cystic mass on ultrasound. Except for circumscription and geographic necrosis, BLBC shares a lot of histologic features with medullary carcinoma. The tumor is usually grade III invasive ductal carcinoma with focal/absent in situ component, high nuclear grade, absence of tubular formation, and high-mitotic rate. There is usually a dense stromal lymphocytic infiltrate, a solid architecture with pushing borders and areas of

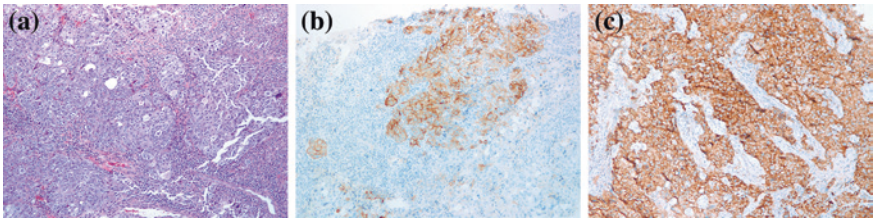

Fig. 11.3 Basal-like breast cancer. **a** H&E stain, 100×. **b** CK5/6 immunostain showing positive staining of the tumor cells. **c** IMP3 immunostain is diffusely positive

geographic necrosis (Fig. 11.3a). Like medullary carcinoma, BRCA-1 associated carcinomas are often BLBC.

Immunohistochemistry

Expression of basal cytokeratins, particularly CK5/6 and CK14 is considered the sine-qua-non of BLBC (Fig. 11.3b). CK17 is also present in approximately 50 % of cases, but may be focal and weak. The expression of EGFR in BLBC varies in several studies, ranging from 45 to 70 %. Since more than 80 % of BRCA-1 associated cancers cluster in the basal-like category, it is not surprising that basal CKs and EGFR expression are also observed in BRCA-1 associated breast cancers. However, attempts to use these markers together with hormone receptors to predict mutation status in these patients has not been successful due to the high overlap between both BRCA-1 and non BRCA-1 associated BLBC [26]. Various other immunohistochemical markers have been studied as a tool to recognize and further characterize this specific subset of tumors. Insulin-like growth factor-II mRNA-binding protein 3 (IMP3), which was first introduced as a marker of aggressive behavior in renal cell and urothelial carcinomas [27], has been demonstrated in 78 % of TNBC, and correlates with CK5/6 expression (Fig. 11.3c) [28]. C-KIT has been reported in approximately 45 % of BLBC. P53 over-expression is also more common in BLBC compared to all breast cancers. Vascular endothelial growth factor (VEGF), maspin, osteopontin, integrin β4, caveolin1 and 2 have all been reported to be preferentially expressed in BLBC [29–33]. However, the only IHC signature of BLBC that has been validated by expression profiling demonstrates that a panel composed of ER, HER2, CK 5/6, and EGFR can identify these tumors with 100 % specificity and 76 % sensitivity [34].

Molecular Characteristics

The literature has been enriched by many studies that focus on better understanding of the molecular background of BLBC, in attempt to translate this molecular

phenotype into targeted therapy. TP53 mutation has been identified in up to 83 % BLBC cases. The mutation is thought to be an early event in tumorigenesis and is related to poor prognosis and resistance to chemotherapy [35, 36]. The link with BRCA-1 gene mutation is well established. More than 80 % of BRCA-1 associated cancers cluster in the basal-like category [37], and many sporadic BLBC were shown to have altered BRCA1 activity and loss of function. Approximately 10–20 % of BLBC show methylation of gene promoter, and some have decreased BRCA1 mRNA. X-chromosome abnormalities, including defects in inactivation, were also identified. Additionally, the dominant-negative transcriptional regulator ID4 has been shown to regulate *BRCA1* expression and to be preferentially expressed in BLBC [38–40]. Additionally, the loss of one *TP53* allele in mice with mammary-specific deletion of *BRCA1* dramatically accelerates mammary tumorigenesis, suggesting that TP53 mutations may act synergistically with BRCA1 defects in sporadic BLBC to drive tumor initiation.

The high-mitotic index and high rates of proliferation that characterize BLBC reflect the expression of several proliferation-related genes. EGFR is expressed in a large percentage of BLBC. A recent study on IMP-3 in BLBC showed it to be the effector of EGFR-mediated tumor migration and invasion suggesting a mechanism by which IMP-3 may be regulated in breast cancer. Cyclin E1 over-expression has also been shown in BLBC. ELISA studies reveal a three-fold increase in VEGF expression levels in TNBC compared to non-TNBC. Moreover, high VEGF-receptor2 expression was observed in a subset of TNBC and correlates with a shorter survival. The expression of Fascin, an invasion promoting gene, was observed in 54 % of BLBC, and in 83 % of BRCA1-associated carcinomas.

Prognosis

TNBC has gained a bad reputation as a tumor of poor prognosis largely because the terms TNBC and BLBC are often incorrectly used as synonyms. Studies have proven that this is not necessarily the case, and using the term TNBC to imply a badly behaving tumor will expose many patients to unnecessary treatments with ample side effects. Members of this diverse family of tumors behave differently and have variable prognoses.

Perhaps the most “innocent” member in this family is ACC. Despite its triple negative nature and paucity of treatment regimens, ACC is considered a low-grade carcinoma with an excellent prognosis. The data from the Surveillance, Epidemiology and End Results (SEER) program show that the 5-year, 10-year, and 15-year survival for patients with mammary ACC are 98, 95, and 91 %, respectively [5]. Many cases are treated with lumpectomy, but simple mastectomy is generally curative. Axillary dissection is unnecessary except for the very rare cases of nodal metastases. Local recurrence is rare, and is usually related to incomplete excision.

Prognostic data on patients with metaplastic carcinomas is somewhat limited due to the uncommon nature of the disease, and have been based largely on patients

treated by mastectomy with axillary dissection. It is unclear if the type and amount of metaplasia has a significant effect on prognosis. However, specific subtypes such as low-grade adenosquamous carcinoma, have a good prognosis compared to other types of metaplastic carcinoma. On the contrary, recent data suggests that Claudin-low carcinomas may have a lower response rate to conventional chemotherapy and a worse clinical outcome than other metaplastic carcinomas [13].

With approximately 38 % mortality rate in the first 2 years, pleomorphic carcinoma has a very poor prognosis [17]. Conversely, secretory carcinoma has a favorable prognosis, especially in children and adolescents. In older patients, the tumor may take a more aggressive clinical course with late metastases [41]. Axillary lymph node and distant metastases are very rare and usually manifested in older patients.

Medullary carcinoma, when defined by strict morphologic criteria, also has a favorable prognosis. This may be related to the intense lymphocytic infiltration that represents the host immune response, the well circumscription that makes resection with wide clear margins a relatively easier task for surgeons, and the high mitotic rate that makes these tumors very sensitive to chemotherapy. Gene expression profiling studies have demonstrated that the expression levels of immune response genes are independent predictors of the outcome in patients with highly proliferative breast cancers. This suggests that the relatively good prognosis of tumors with medullary features may be attributed to the prominent lymphoplasmacytic stromal response. The 10-year survival for patients with pure MC is greater than 80 % in some reports. Axillary lymph node metastases are uncommon and, when present, are usually in fewer than four lymph nodes. However, patients with tumors larger than three cm or those with metastases to more than four lymph nodes do not have the same favorable prognosis. Additionally, patients with BRCA1 mutations who develop MC do not have the same prognosis as those without the mutation. The low level of reproducibility in diagnosing MC, and the concern for under calling an aggressive BLBC tumor as a MC, has led to a decrease in the number of reported MC cases and a marked shrinkage of this controversial subtype. Currently, it is a common practice to treat MC in a similarly aggressive fashion as BLBC.

BLBC represents the “ugly face” of TNBC. It is well-documented now that BLBC has the worst behavior amongst breast cancers. This poor prognosis may be attributable to the over expression of genes promoting proliferation, angiogenesis, and migration. Studies have shown a decreased disease-free survival and overall survival compared to other types of breast cancer. Patients with BLBC are at a higher risk for early relapse/recurrence. A large, central fibrotic scar, occasionally seen histologically, was suggested as a poor prognostic feature, associated with a higher risk of distant metastases [42]. Interestingly, BLBC has a different pattern of distant metastases. Brain metastases, which in itself carry a poor prognosis, are more common among patients with BLBC [43]. The expression of basal cytokeratins in breast cancer has been shown to be associated with a poor outcome [44]. Further, expression of these cytokeratins in node-negative breast carcinoma, is a poor prognostic factor, independent of tumor size and grade [44]. Multivariate analysis indicates that EGFR is also a significant, independent prognostic factor in breast cancer patients, whose expression is associated with shorter disease-free survival [45].

Of all the TNBC subtypes, treatment for BLBC remains the greatest challenge because of its clinically aggressive nature and limited therapeutic options. Traditionally, oncologists have used anthracycline and paclitaxel to treat these breast cancer patients. Even though neoadjuvant chemotherapy results in complete pathologic response in 15–25 % of BLBC [46], most patients continue to have residual disease and remain at a high risk for relapse and death within the first 5 years of diagnosis. Moreover, the nonspecific cytotoxicity of these agents may result in significant, dose-limiting side effects. Thus, the development of targeted therapies with improved therapeutic indices is of paramount importance. One approach was to explore platinum based chemotherapy agents (carboplatin, cisplatin, etc.). Platinum agents produce DNA cross-links, leading to DNA double-strand breaks, normally repaired by BRCA. Since many BLBC exhibits BRCA-1 gene defects, these cells become highly sensitive to the apoptosis induced by these agents. Cisplatin also promotes apoptosis in BLBC by disrupting a complex in the TP53 family that is present selectively in BLBC with mutant TP53. In a recent study, 22 % of patients with TNBC showed complete pathological remission with single-agent neoadjuvant cisplatin [47]. This rate is similar to that observed with non-platinum agents. Platinum agents appear to be the most promising therapy that may improve survival in BLBC.

BRCA1 pathway dysfunction is also the basis for treating BLBC with Poly (ADP) Ribose Polymerase Inhibitors (PARP-I). PARP is involved in base excision repair; an important pathway in the repair of single-strand breaks in DNA [48]. Single-strand breaks become double-strand breaks at replication forks, creating more DNA lesions to be repaired by homologous combination in the absence of functioning PARP. This occurs without increasing or affecting the process of homologous recombination [49]. Combined with the effects of BRCA-1 gene mutations on homologous recombination, increased numbers of DNA errors may lead to a cell cycle arrest and, potentially, permanent arrest and apoptosis in tumors. Cell lines with BRCA dysfunction have been proven to be extremely sensitive to PARP-I [50]. PARP-I are relatively nontoxic compared to general cytotoxic chemotherapy because they do not directly damage DNA, therefore, targeting cooperative pathways that may lead to the development of specific and less toxic therapy. Depending on whether the tumor is due to a BRCA germline mutation, or a sporadic mutation with BRCA-like effects, normal tissue outside the tumor maintains at least one copy of wild type BRCA, thus enabling the repair of normal cells affected by the PARP inhibition [50]. This approach uses the concept of *synthetic lethality* by targeting DNA repair pathways in a complementary manner, leading to a lethal combination [51].

Aberrant VEGF pathway activation shown in BLBC has led to the investigation of targeting anti-angiogenic therapeutic strategy to VEGF and its downstream receptors. Bevacizumab, the anti-VEGF antibody, was shown to increase disease-free survival when combined with paclitaxel in patients with TNBC by four months, compared to paclitaxel alone. However, the overall survival was unaffected [52]. Other small-molecule multikinase inhibitors have been developed as possible anti-angiogenic agents. These inhibit VEGFR and other receptor tyrosine kinases

[53]. Sunitinib (Sutent®) is a multi-targeted receptor tyrosine kinase inhibitor. Sunitinib inhibits cellular signaling by targeting multiple receptor tyrosine kinases (RTKs), including platelet-derived growth factor (PDGF-Rs) and VEGFRs, which play a role in both tumor angiogenesis and tumor cell proliferation. This simultaneous inhibition leads to reduced tumor vascularization, cancer cell death, and ultimately tumor shrinkage. Sunitinib was shown to induce an 11 % response rate when used as a single agent in patients with previously treated metastatic breast carcinoma. Fifteen percent of BLBC patients responded to treatment [54]. Other studies have demonstrated a response in one third of patients with metastatic or locally advanced BLBC to treatment with sunitinib added to paclitaxel. Other VEGFR multikinase inhibitors have not been as promising [55]. The currently demonstrated limited response to antiangiogenic agents is considered disappointing.

Since EGFR is upregulated in the majority of BLBC, it represents a potential therapeutic target. Lapatinib is a dual inhibitor of EGFR and HER2 tyrosine kinases [56]. In randomized trials, the use of lapatinib with placitaxel was shown to have a significant benefit in HER2-amplified tumors [57]. In contrast, patients with HER2-negative tumors and overexpression of EGFR did not benefit from the addition of lapatinib [56]. This suggests that although EGFR overexpression is present in the majority of BLBC, it may not be a helpful therapeutic target.

Multiple other downstream kinases are under consideration as targeted therapy for BLBC patients. Constitutive activity of these pathways downstream from EGFR may be an explanation for the lack of response to EGFR-targeted therapies. One such target currently being explored is the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) kinase (MEK), a signaling pathway with a central role in promoting tumor initiation and progression [58]. Activation of this pathway has been shown to be associated with an increased risk of metastasis in breast cancer patients [59]. As a therapeutic target, early clinical studies have demonstrated a limited response [60, 61]. A more recent study has shown that BLBC appears to be particularly sensitive to MEK inhibitors. This study also elucidated the interaction and potential negative feedback loop between the MEK cascade and the phosphoindositide 3-kinase (PI3K)-PTEN-AKT signaling cascade, which counteracts the effects of MEK inhibition of cell cycle and apoptosis induction. These findings may, in part, explain why initial studies showed only a modest response to MEK inhibition and suggest concurrent treatment with both MEK and PI3K inhibitors is a promising therapeutic possibility [58].

Summary

TNBC is a heterogenous group of breast carcinoma with varying morphology, immunophenotype and molecular characteristics. Treatment and prognosis are highly variable amongst the group. Morphologic correlation with immunohistochemistry and molecular signature is the key to establish an accurate diagnosis, which will then dictate further management.

Key Points

- TNBC is a heterogeneous group of tumors accounting for about 10–15 % of all breast cancers.
- ACC is a low-grade carcinoma, morphologically identical to its salivary gland counterpart and has excellent prognosis.
- Claudin-low associated tumors are considered subsets of metaplastic carcinoma with low expression of GATA3-regulated genes and genes responsible for cell-cell adhesion.
- Carcinomas with apocrine differentiation have characteristic morphology and immunophenotype (GCDFP and AR positive), but do not represent a distinct molecular entity.
- Pleomorphic carcinomas are poorly differentiated tumors with highly pleomorphic, bizarre cells, and tumor giant cells mimicking sarcoma. These tumors have very poor prognosis.
- Secretory carcinoma has a favorable prognosis in children and young adults but can be aggressive in older patients.
- More than 80 % of BRCA-1 associated cancers cluster in the basal-like category.
- A panel of ER, HER2, CK 5/6, and EGFR can identify BLBC with 100 % specificity and 76 % sensitivity.
- MC are now commonly treated as BLBC due to the low level of reproducibility in its diagnosis and concern for under-treating an aggressive BLBC.

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