Chapter 6

Prognostic Biomarkers for Breast Cancer Metastasis

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Abstract

Breast cancer treatment has improved rapidly through the years, starting from surgery, to hormonal therapy, to targeted therapy. Despite this, tumor metastasis remains the highest cause of breast cancer–related death. The current regime to deter metastasis is through adjuvant therapy, but such therapy frequently yields undesirable side effects. As such, prognostic markers for metastasis are important to stratify patients for adjuvant therapy so as to ameliorate the standard of living of patients with low metastatic potential. So far, only a few well-characterized prognostic biomarkers for metastasis are used in clinics. This chapter will cover both established and novel prognostic biomarkers for breast cancer metastasis and metastatic breast cancer prognosis. The potential of using these biomarkers as predictive biomarkers or new targeted therapy will also be discussed.

Keywords: metastasis, prognostic biomarker, metastatic breast cancer, relapse, recurrence, distant-free metastasis survival, overall survival

1. Introduction

Breast cancer remains the most frequently diagnosed cancer in women worldwide. In the United States (US), the American Cancer Society estimates that in 2018, the highest frequency of cancer diagnosed and the second highest cancer-related death in women will be breast cancer, at 30 and 14%, respectively [1]. Breast cancer survival statistics have improved tremendously over the years with a decrease of 39% mortality from 1989 to 2015 [1, 2]. This is mainly due to mammogram screening resulting in early detection and intervention [3, 4]. When diagnosed at a localized stage, the 5-year survival rate is 99% [5]. However, metastasis remains the major cause of mortality in breast cancer patients. Five-year survival rate decreases dramatically according to spread of cancer, with regional and distant metastasis

spreads at 85 and 27%, respectively [1]. Ten-year survival rate for stage IV metastatic breast cancer female patients is only approximately 13% [6]. These statistics indicate that metastasis is the major barrier against breast cancer eradication.

Breast cancer can be categorized largely into two types, *in situ* and invasive breast cancer [2]. *In situ* represents the subset in which the cancer is still confined within the transformed origin. Ductal carcinoma *in situ* (DCIS) and lobular carcinoma *in situ* (LCIS) are the two main types of frequently diagnosed *in situ* breast cancer at 83 and 13%, respectively [2]. DCIS, as its name suggests, refers to cancer originating from the epithelial cells of the breast ducts, whereas LCIS arises from the lobules of the breast. On the other hand, majority (80%) of breast cancer will become invasive, i.e., that they will outgrow into surrounding breast tissue [2].

The primary treatment for *in situ* breast cancer is surgery. This includes lumpectomy where only the tumor and surrounding tissues are removed or mastectomy in which the entire breast is removed [2]. Very often, radiation and adjuvant therapy are recommended after surgery to prevent recurrence and to eliminate breast tumor cells which might have spread [2]. Examples of adjuvant therapy are cytotoxic chemotherapy, hormone therapy, and targeted therapy [2, 3]. These will be discussed in more detail in the later sections.

Although adjuvant therapy has been shown to be beneficial in preventing metastatic recurrence, the patient's quality of life is severely affected in many cases. Side effects include fatigue, osteoporosis, increased thromboembolic events, premature menopause, weight gain, and mild memory loss [7, 8]. Although as many as 80% of patients receive adjuvant treatment, only 40% of them relapse and die from metastatic breast cancer indicating that majority of patients are over-treated and suffer unnecessary side effects [3]. In addition, only 15% of patients treated with tamoxifen after surgery will have distant recurrence, indicating that 85% of patients will be overtreated if chemotherapy is mandatory [9].

One solution to overcome unnecessary treatment is through identifying patients with high and low risk of metastasis using prognostic biomarkers. Current established metastasis biomarkers are available but have poor predictive power [10]. With new concepts such as gene expression profiling and circulating tumor cells, new prognostic markers with greater accuracy could be identified. The following sections will describe established markers as well as new upcoming markers for the prediction of survival, metastasis risk, and recurrence risk for metastatic breast cancer. Current and potential use of these markers in the clinic as predictive biomarkers for treatment and as potential targets is also discussed.

2. Established biomarkers

2.1. Tumor size and lymph node status

The tumor-node-metastasis (TNM) staging system is commonly used to stage breast cancer progression during initial diagnosis [11]. It constitutes a manner to measure the aggressiveness of the cancer. The abbreviations represent different characteristics of the cancer. "T" represents tumor size, "N" indicates the number of lymph nodes that the cancer has spread to,

and "M" conveys the presence of distant metastasis [11]. In the absence of distant metastasis ("M"), tumor size and lymph node status are established prognostic markers for likelihood of metastasis. Specifically, primary tumors that are less than 2 cm have low prognosis for developing into metastatic breast cancer. Tumor sizes of 2–5 cm and more than 5 cm have a high and very high likelihood of progressing into metastatic cancer respectively [12–15]. Likewise, breast cancer patients with no detectable lymph node metastases are at low risk of distant metastasis. Patients with presence of lymph node metastasis have high risk of metastasis, and more than four lymph node metastases represent a high probability progressing to distant metastasis [12–15].

2.2. Histological grade

Histological grade is the determination of how differentiated a tumor is. As the histological grade increases, the tumor appears more poorly differentiated [16]. The determination of histological grade is performed by a trained pathologist using certain characteristics of the cancer tissue section such as mitotic count, extent of tubule or gland formation, and nuclear pleomorphism [16]. Histological grade 1 tumors have low risk of metastasis, while grade 2 and 3 tumors have intermediate- and high-risk tumors [12, 14, 17]. Integrating histological grade with the aforementioned tumor size and lymph node status, several prognostic indices such as the Nottingham Prognostic Index, the Kalmar Prognostic Index, and the St. Gallen guidelines have been established and used in clinics to aid in adjuvant treatment decision [16].

2.3. Angioinvasion

Angioinvasion is the presence of blood vessel invasion by cancer cells. In lymph node negative patients, angioinvasion has some prognostic value in predicting metastasis [18, 19]. In particular, tumor emboli in more than three blood vessels suggest a high risk of metastasis [18, 19]. Although these tumor characteristics (tumor size, lymph node status, histological grade, and angioinvasion) represent a simple and cheap method to predict metastasis, statistics show that these prognostic factors only accurately predict metastatic outcome in 30% of patients [3, 16]. As such, better prognostic markers are required for the remaining 70%.

2.4. Molecular subtype

Invasive breast cancer can be divided into four main molecular subtypes based on the presence of hormone receptors (estrogen or progesterone) (HR) and human epidermal growth factor receptor 2 (HER2) [2]. The four subtypes are luminal A (HR+/HER2−), luminal B (HR+/HER2+), HER2 enriched (HR−/HER2+), and triple negative (HR−/HER2−) at frequencies of 71, 12, 5, and 12% of all invasive breast cancer, respectively [2].

In general, luminal A and B subtypes are associated with the most favorable prognosis and least aggressive, followed by HER2 enriched and triple negative sequentially [2, 20–23]. In a 6.9-year follow-up study on patients who were initially diagnosed with localized breast cancer, the frequency of distant metastasis increases progressively in the following order, luminal A (6.4%), luminal B (12.1%), HER2-enriched (19.2%), and triple negative (27.4%) [24]. Another

study involving metastatic breast cancer found that luminal A exhibited the longest survival rate (34.4 months), followed by luminal B (24.8 months), HER2 enriched (19.8 months), and triple negative (8.8 months) [25]. Similarly, follow-up on patients with early stage breast cancer found that survival with distant metastasis showed similar patterns [26]. Luminal A survived the longest duration (2.2 years), followed by luminal B (1.6 years), HER2 enriched (0.7 years), and triple negative (0.5 years) [26]. These findings strongly suggest that molecular subtype correlates with metastasis rate and survival and can be used as a prognostic marker for metastasis.

In fact, molecular subtypes are already routinely used in clinics as prognostic and predictive biomarkers for overall survival and stratification of patients to targeted therapy [2]. As a predictive biomarker, patients who are either HR+ or HER2+ benefit majorly due to targeted therapy options. The estrogen receptor (ER) in breast cancer plays an important tumor promoting role by activating downstream intracellular signals for proliferation and survival [27]. As such, patients with ER+ breast cancer are routinely treated with selective estrogen receptor modulators (SERMs) [8]. Tamoxifen is the first approved SERM to be used for the treatment of ER+ metastatic breast cancer [8]. Five-year use of tamoxifen as an adjuvant significantly reduced local and distant recurrences by 40–50% making ER status both a prognostic and predictive biomarker [28].

Apart from using SERMs, aromatase inhibitors (AIs) are an alternative targeted treatment for ER+ breast cancer patients. These inhibitors function to block estrogen production by inhibiting the aromatase enzyme [8]. Studies have found that there is no difference in efficacy and time to distant recurrence when compared to tamoxifen treatment [29]. In addition, both tamoxifen and AI treatment are associated with increased overall survival and distant metastasis-free survival [30]. Since ovaries are the main source of estrogen, ovarian surgery, ovarian irradiation, or ovarian suppression by drugs have been shown to improve therapeutic outcomes [2, 31]. Particularly, in a clinical study, 5-year disease free survival was as high as 91.1%, when ER+ premenopausal women were treated with adjuvant ovarian suppression combined with AI treatment [31]. Additionally, use of AI with ovarian suppression significantly decreased recurrence as compared to tamoxifen with ovarian suppression [31]. These evidences strongly illustrate the use of ER status for metastatic survival prognosis and for tamoxifen or AI adjuvant therapy decision.

Historically, HER2-enriched metastatic breast cancer is associated with high aggressiveness and has a poor prognosis [2, 32–34]. That is until the first anti-HER2 targeted therapy Trastuzumab clinical trial emerged, which showed improved clinical outcomes by including Trastuzumab into adjuvant treatment with chemotherapy for HER2-enriched metastatic breast cancer [35]. Trastuzumab is a monoclonal antibody which binds and targets the extracellular portion of the HER2 receptor protein [8]. Specifically, combining Trastuzumab with standard chemotherapy for HER2-enriched metastatic breast cancer resulted in increased time to progression, overall survival, and duration of response [35, 36].

In recent years, many new biologics targeting HER2 have been approved for advanced metastatic HER2-enriched breast cancer [37]. Pertuzumab, another monoclonal antibody which inhibits HER2 dimerization, is approved for use in combination with trastuzumab

in metastatic HER2+ breast cancer [38]. Treatment of HER2-enriched metastatic breast cancer patients using a combination of Pertuzumab, Trastuzumab, and Docetaxel resulted in a significant increase in median overall survival of 15.7 months as compared to just treating with Trastuzumab and Docetaxel [38]. Increase in progression free survival and duration of response by 6.3 months and 7.7 months, respectively, were also noted when Pertuzumab was used in combination [38].

Another recently approved biologic for HER2+ advanced breast cancer is the antibody-drug conjugate T-DM1 [39, 40]. It involves the ingenious exploitation of Trastuzumab's specificity to HER2+ breast cancer cells to deliver the linked cytotoxic microtubule-inhibitory drug DM1 directly to HER2+ cancer cells [39, 40]. It is particularly effective in slowing disease progression for HER2+ advanced breast cancer patients who were initially treated with first line Trastuzumab/Taxane combination [39]. Progression free survival when treated with T-DM1 was significantly longer (9.6 months) as compared to the standard second line treatment (6.4 months) [39]. Overall survival improved significantly from 25.1 to 30.9 months [39]. Additionally, patients treated with T-DM1 experienced less toxicity as compared to the standard second line treatment [39]. Taken together, it is recommended that the Docetaxel/Trastuzumab/Pertuzumab combination be used as a first line choice and T-DM1 as a second line therapy [37].

The importance of HER2-targeted therapy for metastatic breast cancer is further emphasized by the finding that as many as 16% of initially HER2 negative breast cancer exhibits HER2 expression upon metastasis [37]. This indicates that HER2-targeted therapies could be extended to treat metastasis in this select group of patients, and it is recommended in clinics that HER2 status in metastatic cells be tested by fluorescence in situ hybridization or immunohistochemistry staining to evaluate eligibility for HER2-targeted therapy regardless of initial subtype of the primary tumor [37]. Overall, these findings highlight the importance of using HER2 status as both a prognostic and predictive biomarker in clinics for metastatic breast cancer.

As for triple negative cancers which do not currently have their own targeted therapy, neoadjuvant anthracycline-based chemotherapy has been found to benefit this group [41]. Clinical response in triple negative was 85% as compared to luminal (47%) or HER2 positive (70%), and all subtypes had equally good prognosis after treatment [41]. However, this only applies to triple negative patients who exhibited pathologic complete response from treatment, which constitutes only 27% [41]. As such, for majority of triple negative patients who do not display complete pathologic response after chemotherapy, more studies need to be done to discover targets specific against triple negative breast cancer.

In addition to metastasis frequency and survival, molecular subtypes could potentially predict distant metastasis tumor sites and distant relapse sites. With the exception of triple negative basal subtype, bone metastasis is the most common metastasized site among all subtypes, with the luminal subtypes displaying the highest frequency [26, 42]. Correspondingly, bone is the most frequent metastatic relapse site, with luminal subtypes exhibiting the highest frequency [43, 44]. The subtypes with the highest brain and lung metastasis rates are HER2 enriched and triple negative [26]. Among all subtypes, metastatic lung and brain relapse are

highest for triple negative [43, 44]. HER2-enriched subtype has the highest liver metastasis rate, whereas triple negative has more distant lymph node metastasis as compared to other subtypes [26, 42]. The importance of determining site of metastasis is covered in the next section.

2.5. Site of distant metastasis

The significance of predicting site of metastasis for metastatic breast cancer patients is highlighted by the intrinsic correlation with overall survival and survival after distant recurrence. In the following order, breast cancer patients with single site brain, lung or liver, and bone metastasis have the worst to best prognosis [42, 45]. Median overall survival rates for patients with brain, lung, liver, and bone metastasis are 11 months, 30 months, 31 months, and 41 months, respectively [42]. In addition, the survival trend holds true when patients are stratified based on HR indicating that it is independent of HR status [45].

Postmetastasis distant recurrence is also associated with the site of recurrence. In particular, first visceral (including brain) site recurrence is associated with a poorer prognosis as compared to first bone recurrence with 3-year breast cancer specific survival (BCSS) rate at 13 (visceral) and 23% (bone), respectively [46]. When compared to recurrences closer to the primary tumor, first local and first lymph node recurrences 3-year BCSS are significantly higher at 83 and 33%, respectively, indicating that metastatic site proximity to primary tumor origin site is also strongly linked to prognosis [46].

Apart from the site of distant metastasis, the number of initial metastatic sites is also prognostic of survival. Patients with multiple metastatic sites have significantly poorer overall survival than patients with single metastatic site in both HR+ (9 months) and HR− (5 months) patients [45]. Multiple metastatic sites are also more prone to occur in HR− patients, which are in line with the poorer prognosis of HR− patients [45].

2.6. Age of diagnosis

Indubitably, as with many diseases, age is a major determinant of prognosis in metastatic breast cancer. Survival rate in stage IV invasive breast cancer patients significantly decreases with age [6]. Ten-year breast cancer specific survival rates for three groups of stage IV patients namely, below the age of 40 years, between 41 and 50 years, and between 51 and 70 years, drops from 15.7 to 14.9% to 11.7%, respectively [6]. Likewise, another study found similar trends in metastatic breast cancer patients, where overall survival decreases significantly with age, from 32 months (age < 50 years) to 25 months (50–69 years) to 16 months (>69 years) [47]. One plausible explanation is that younger patients are more physically fit to endure treatment than elder patients, and this is supported by a significantly higher rate of surgery and radiation therapy underwent by patients below 69 years [47].

Age is also a determinant in the prediction of distant metastasis site. In accordance with the age-related survival trend, frequency of the deadlier lung metastasis increases significantly with age from 5.9% (age < 50 years) to 7.6% (50–69 years) to 14.2% (> 69 years), respectively [47]. Correspondingly, a significantly lower rate of the less lethal distant lymphatic metastasis

is observed as age increases with rates at 7.3, 5.4, and 4.0% for patient age of less than 50 years, 50–69 years, and more than 69 years, respectively [47]. Metastasis to the brain, liver or bone is not dependent on age [47]. Interestingly, multiple metastatic sites, which are associated with poorer prognoses, occurred more frequently in younger patients (<69 years) than in older patients (>69 years) at approximately 34.9 (age < 50 years) and 36.2% (50–69 years), and 28.3% (>69 years), respectively [47]. This discrepancy could be explained by the higher rate of treatment in younger patients, suggesting that patients with multiple metastatic sites could benefit from surgery and radiation therapy [47]. Overall, age at diagnosis is a strong independent prognostic factor for metastatic breast cancer patient survival and for predicting the site of metastasis [47].

2.7. Urokinase-type plasminogen activator (uPA) and plasminogen activator type 1 inhibitor (PAI-1)

The urokinase-type plasminogen activator (uPA), which is a serine protease, and its inhibitor plasminogen activator type 1 inhibitor (PAI-1) are involved in the degradation of extracellular matrix, which is a crucial process in the initial stages of metastasis [48]. Although PAI-1 inhibits uPA activity, it has been found to promote tumor invasion and angiogenesis through other means [49]. As such, both uPA and PAI-1 could potentially be used as metastasis prognostic markers. Supporting this, high uPA and PAI-1 levels are correlated with lower metastasis-free survival and overall survival in breast cancer patients [50–54]. In addition to being a prognostic marker, both uPA and PAI-1 could be used as predictive biomarkers for adjuvant therapy. In a study, patients with high uPA and PAI-1 levels benefited significantly from adjuvant chemotherapy compared to patients with low uPA and PAI-1 levels [55]. This indicates that patients with high uPA and PAI-1 levels could be treated with chemotherapy after surgery.

Furthermore, since uPA functions by binding to its receptor, urokinase plasminogen activator receptor (uPAR), the interaction could be exploited for metastasis targeted therapy. Indeed, one of the developments in this area is the use of an antibody to target uPAR [56, 57]. Remarkably, the antibody is shown to inhibit invasion of cancer cells and induce apoptosis, indicating its potential use for metastatic breast cancer [57].

2.8. Gene expression profiling

With the advent of gene expression profiling, treatment options are expected to shift toward a more personalized approach [58]. Although the idea of sequencing every cancer patient for individualized prognosis and treatment remains elusive, using multigene signatures to stratify patients into groups with different prognosis and therapeutic options has been very well established and routinely used in clinics. The first report of using high throughput methods for stratification of patients started in diffuse large B-cell lymphoma (DLBCL) patients [59, 60]. Based on their gene signatures from microarray, two subtypes of DLBCL, namely germinal center B-like DLBCL and activated B-like DLBCL, were characterized and were prognostic of overall survival [59]. In fact, molecular subtypes of breast cancer (mentioned in previous sections) were also identified using microarray-based gene expression profiling and are routinely used in clinical settings for prognosis [20–23].

Following the clinical success of using multigene signatures to identify and stratify patients with different clinical outcomes, two different gene expression profiling panels have emerged and are currently used in clinical settings. Each platform relies on different gene panel and is routinely used for predicting metastasis risk, local and distant metastasis recurrence, and for treatment decisions [61]. These are Oncotype DX and MammaPrint. Other gene expression profiling panels such as the PAM50 [62], two-gene expression ratio [63], and MapQuant DX [64] are not covered here [65].

Oncotype DX is the most widely used multigene panel tool for the prediction of distant recurrence risk in the United States [2]. It is a reverse-transcription-polymerase-chain-reaction (RT-PCR) based assay which measures the expression of 16 cancer-related genes and 5 reference genes in tumor tissue [9]. Based on the expression level of the 21 genes, an algorithm computes a recurrence score which quantifies the probability of distant recurrence [9]. Using the recurrence score, a patient with higher score is considered high risk and would likely benefit from chemotherapy as compared to a patient with lower score who could avoid chemotherapy altogether [2, 9].

Oncotype DX is currently utilized in clinics to predict distant recurrence for ER+, lymph node negative breast cancer patients who had prior tamoxifen treatment [9]. In the original paper, 51% of ER+, lymph node negative, tamoxifen-treated patients were classified under low-risk, and indeed, only 6.8% of this group had distant recurrence within 10 years [9]. This is in comparison with the high-risk group consisting of 27% of patients who had a 30.5% distant recurrence rate within 10 years [9]. In addition, recurrence score could also predict overall survival and relapse-free survival [9]. In support, a recent prospective validation study showed that Oncotype DX could potentially select low recurrence patients with high probability to forgo chemotherapy [66]. Five-year recurrence free rate from all sites and distant site, for patients with low recurrence score and only underwent tamoxifen treatment, were a high 98.7 and 99.3%, respectively [66]. Overall survival and invasive disease-free rates were up to 98 and 93.8%, respectively [66]. These findings show the clinical applicability of Oncotype DX to select for patients who could forgo chemotherapy and its unnecessary side effects.

The second most commonly used multigene panel for prognosis and treatment decision is MammaPrint [65, 67]. It utilizes an oligonucleotide microarray to measure the expression of 70 genes to identify gene signatures that stratify patients into a “good” or “poor” prognosis that predicts metastasis risk in lymph node negative early breast cancer patients [68–72]. Sensitivity and specificity of MammaPrint are 91 and 73%, respectively [3]. Genes involved in “poor” prognosis signature include angiogenesis, cell cycle, invasion, and metastasis [69].

In one of the earlier studies depicting the prognostic value of MammaPrint patient stratification, 10-year distant metastasis free probabilities were lower for “poor” prognosis group at 50.0% than in “good” prognosis group at 85.2% [68]. Overall survival rates were also significantly different between groups at 50.6 and 85.2% for “poor” and “good” prognosis groups correspondingly [68].

In a follow-up study with longer term survival statistics, 25-year distant metastasis free survival was significantly lower for “poor” prognosis group (41.6%) as compared to “good”

prognosis group (60.4%) [72]. Overall survival at 25 years also showed the same trend at 44.5 and 57.3% for "poor" and "good" prognosis groups, respectively [72]. These statistics show the relevance of using MammaPrint in clinical settings as a prognostic biomarker for metastasis risk and overall survival. It could also be applied as a predictive biomarker for selecting patients with "poor" prognosis for adjuvant treatment.

Overall, the importance of multigene expression profiling tools for prognosis and treatment decision in the clinic is apparent when compared to classical clinicopathological parameters which are determined by a trained pathologist. It is found that low agreement exists among pathologists in breast cancer grading as tumor grading includes a degree of subjectivity [9]. A study comparing the different gene expression profiling tools found that although different gene sets were used among different panels, 4 out of 5 (including Oncotype DX and MammaPrint) achieved high concordance in relation to predicting outcomes [73]. As such, tools like Oncotype DX and MammaPrint stand out in this aspect.

3. New biomarkers

3.1. Improved gene expression profiling

Although the first generation gene expression profiling tools, Oncotype DX, and MammaPrint have greatly advanced the prognosis of breast cancer patients for metastasis risk and distant recurrence, a major drawback is that they are unable to accurately predict late distant recurrence of more than 5 years [74]. Two newer gene expression profiling tools, EndoPredict and The Breast Cancer Index, have emerged successful in this aspect [74].

EndoPredict is a RT-PCR-based assay, which measures the expression of eight cancer genes and three housekeeping genes to stratify patients into high- and low-risk distant recurrence groups [75–77]. A newer version of it combines the 11 gene expression with tumor size and nodal status to calculate a risk score termed the EPclin [76]. Patients with an EPclin score of less than 3.3 are classified as low-distant recurrence risk, while more than or equals to 3.3 are classified as high-distant recurrence risk [76]. The EPclin score is the best predictor of late relapse (>5 years), when compared to the earlier version of EndoPredict or to nodal status and tumor size alone [76]. Metastasis free survival for short term (less than 5) and long term (5–12 years) are also significantly different between the EPclin-stratified high- and low-risk groups, validating its applicability to predict metastasis [76]. In addition, distant recurrence free rates at 10 years in EPclin low-risk group is higher than in EPclin high-risk group, at 98.20 and 87.69%, respectively, depicting its ability to predict distant recurrence for longer time frames [76].

The breast cancer index (BCI) is another RT-PCR-based assay, which combines two independent biomarkers namely a set of five cell cycle genes and the HOXB13 and IL17BR gene ratio to determine the recurrence probability of early stage ER+, lymph node negative breast cancer patients [63, 78, 79]. Independently, both the five gene panel and the two gene ratio are associated with distant metastasis free survival rates [78]. However, when combined, three

groups could be formed, low-, intermediate-, and high-risk groups, which are significantly predictive of metastasis occurrence [78]. Ten-year distant metastasis free survival for low-, intermediate-, and high-risk groups are 98, 87, and 60%, respectively [78]. Additionally, in a study comparing the prognostic ability of BCI with Oncotype DX and another gene panel, BCI emerged as the only test capable of significantly predicting both early and late distant metastasis recurrence, whereas the other two were only able to predict early recurrence [80].

In general, both EndoPredict and BCI seem to be superior as compared to the first-generation counterparts. This is particularly in terms of predicting longer term distant recurrence while also predictive of early recurrence [74].

3.2. Circulating tumor cells

An essential part of distant metastasis requires cells from the primary tumor to migrate into the bloodstream to spread throughout the body till it finds a secondary site to establish a secondary tumor [3]. As such, it is not surprising that circulating tumor cells (CTCs) in peripheral blood could be utilized as a prognostic indicator. The peripheral blood also represents an easily accessible region, which is an added advantage of using CTCs for prognosis [81].

The history of CTCs dates back as far as the nineteenth century when researchers have just begun to study the concept of tumor cells shedding from primary tumor [82, 83]. Today, there are multiple platforms for the isolation and detection of CTCs in the peripheral blood in clinical use [84]. Termed the "golden standard", the CellSearch system is the only FDA-approved platform for such purpose in breast, prostate, and colorectal cancer [84]. The problems associated with detection of CTCs are its rare amount in the peripheral blood and the absence of a universal surface marker for different cancer cell types [84]. The CellSearch system overcomes these sensitivity and specificity issues through the use of an antibody to capture CTCs and poststaining the captured CTCs for identification [84]. Specifically, an avidin-biotin anti-EpCAM antibody complex is used to bind CTCs followed by a magnetic capture to isolate CTCs [84–86]. Following which, the captured pool of cells is stained with DAPI and cytokeratins CK8, CK18, and CK19 to select for nucleated and epithelial cells, respectively [84–86]. Additionally, to differentiate from circulating white blood cells, anti-CD45 is used to further isolate CTCs [84–86]. Other systems using size [87–89], density [90], and microfluidic [91] characteristics of CTCs for isolation exist but are not covered in this chapter [81].

Enumerating CTCs in peripheral blood holds immense potential in clinics. The early paper using the CellSearch system to study progression of metastatic breast cancer provided many useful information [86]. The first thing noted was that CTCs were only present in metastatic breast cancer patients. Two or more CTCs per 7.5 mL of blood were present in metastatic breast cancer patients, while CTCs were rare (less than or equal to 1) in both healthy and benign breast cancer women [86]. Next, CTCs were independent prognostic marker of overall survival and progression-free survival [86]. After new treatment, patients with high level of CTCs (CTCs $\geq$5) had a significantly lower median progression-free survival and overall survival than patients with low level of CTCs (CTCs <5), at 2.1 months versus 7.0 months for progression-free survival and 8.2 months versus >18 months for overall survival, respectively [86]. Additionally, the prognostic value of CTCs for overall survival and progression-free

survival is also validated in two other studies, wherein one of it is a prospective study with metastatic breast cancer patients who were not treated previously [92, 93].

In terms of treatment, median progression-free and overall survival differed significantly between patients with CTCs ≥5 before treatment and CTCs <5 after treatment (1st group) and patients with decrease in CTCs after treatment but still ≥5 after treatment (2nd group) [86]. Median overall survival and progression free survival for 1st group are 7.6 months and 14.6 months, and for the 2nd group, 2.1 months and 9.2 months, respectively [86]. This suggests that CTCs could potentially be used to measure treatment efficiency, although the authors cautioned against this interpretation [86]. Following this, two other studies have also observed CTCs as a predictor of therapy efficiency for metastatic breast cancer [94, 95].

3.3. TIP60

Tat-interactive protein 60 kDa (TIP60) is a haploinsufficient tumor suppressor involved in both early and late stage breast cancer [96–99]. In particular for late stage progression, TIP60 is known to regulate epithelial to mesenchymal transition, an important pathway for cellular metastasis [96, 97]. Both *in vitro* and mouse models have shown that loss of TIP60 results in increased metastatic breast cancer cell migration and invasion, indicating that therapies to increase TIP60 in breast cancer could be a potential therapeutic approach for metastatic breast cancer [96, 97]. Additionally, the microRNA miR-22 inhibits the expression of TIP60, thereby making it a promoter of metastasis and a potential therapeutic target for metastatic breast cancer [97]. More importantly, both miR-22 and TIP60 are prognostic of overall survival and metastasis free survival [96, 97]. As such, both miR-22 and TIP60 expression levels would be invaluable tools in clinics to predict metastatic probability and prognosis of overall survival.

4. Future directions/conclusions

Using only clinicopathological characteristics for assessing metastasis risk, the St Gallen criteria and National Institutes of Health criteria each classified a low 15 and 7% of lymph node negative breast cancer as low metastasis risk, respectively [3, 68]. However, after 10 years, up to 25% of the low-risk group developed distant recurrence [3, 68]. In addition, only slightly less than half (45%) of high-risk patients developed metastasis, indicating that the remaining 55% of "high-risk" patients had adjuvant treatment and tolerated its unnecessary effects [3, 68]. In contrast, using MammaPrint in the same cohort, a high 60% of total patients were categorized as low risk, out of which only 13% of these low-risk patients developed metastasis after 10 years, showing that as many as 52.2% of overall patients were safely spared from adjuvant therapy, as compared to 5.25 to 11.25% of overall patients stratified using clinicopathological characteristics [3, 68]. These findings clearly delineate the superiority of gene expression panels for prognosis as compared to just clinicopathological characteristics.

A more beneficial solution would be to combine both clinicopathological markers and multigene expression profiling to have an additive or synergistic effect in prediction for the betterment of patient prognosis and prediction of treatment outcomes. An example is the multigene

expression EPclin risk score which combines its predecessor, the EndoPredict with tumor size, and nodal status to better predict distant metastatic recurrence as compared to if the markers were used individually [76].

However, a significant problem still exists in the field of prognosis for metastatic breast cancer. Many of the gene expression profiling tools, such as Oncotype DX and BCI, are suitable only for prediction of distant metastasis recurrence in ER+, lymph node negative metastatic breast cancers [74, 100]. As such, it represents a gap in the identification of prognostic markers for other subtypes. For this, newer markers like the detection of CTCs which does not discriminate between subtypes may be used. TIP60 which also does not discriminate between subtypes in risk-free survival rates [98] may be explored and could potential be used as a prognostic biomarker for breast cancer metastasis. Other upcoming biomarkers which are not discussed here such as blood-based biomarkers [101] and long noncoding RNA [102] also holds immense potential as prognostic markers in breast cancer metastasis.

Overall, the field of metastatic breast cancer prognosis has come a long way, beginning with clinicopathological markers to molecular subtypes to multigene expression profiling and eventually CTCs. Continuing on, it is likely that future direction for this field will entail combining existing biomarkers together or with newly identified biomarkers, leading to tremendous improvements in metastatic breast cancer prognosis and in predicting metastasis.

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