Review

Molecular biology of breast cancer metastasis
Inflammatory breast cancer: clinical syndrome and molecular determinants

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Abstract

Inflammatory breast cancer (IBC) is an aggressive form of locally advanced breast cancer (LABC) that effects approximately 5% of women with breast cancer annually in the USA. It is a clinically and pathologically distinct form of LABC that is particularly fast growing, invasive, and angiogenic. Nearly all women have lymph node involvement at the time of diagnosis, and approximately 36% have gross distant metastases. Despite recent advances in multimodality treatments, the prognosis of patients with IBC is poor, with a median disease-free survival of less than 2.5 years. Recent work on the genetic determinants that underlie the IBC phenotype has led to the identification of genes that are involved in the development and progression of this disease. This work has been aided by the establishment of primary human cell lines and animal models. These advances suggest novel targets for future interventions in the diagnosis and treatment of IBC.

Keywords: inflammatory breast cancer, insulin-like growth factor-binding proteins, molecular genetics, RhoC GTPase

Introduction

IBC is a form of LABC that was first described by Lee and Tannenbaum in 1924 [1]. The unique clinical and pathologic syndrome and discouraging outcome make IBC a very distinct form of breast carcinoma. Three biologic features make IBC unique. First, it is rapidly progressive, being the most lethal type of LABC. Second, it is highly angiogenic and angioinvasive. Third, its aggressive behavior and angiogenicity are intrinsic characteristics of the tumor, and are present from its inception. The angiogenicity and propensity to invade vessels confer to IBC an extremely high metastatic potential.

The designation 'inflammatory' stems from the clinical appearance, which mimics an acute inflammation of the breast. IBC is a clinical diagnosis. Haagensen [2] required at least one-third of the breast to be involved with 'grave signs' of breast cancer to make a diagnosis, but others have shown that patients with less than one-third involvement of the breast have a similarly poor prognosis [3].

Pathologists rely on the finding of dermal lymphatic involvement to confirm the clinical diagnosis, and a skin biopsy should be performed when there is clinical suspicion of IBC. Although in many patients the clinical and

EGFR = epidermal growth factor receptor; ER = estrogen receptor; HME = human mammary epithelial (cell); IBC = inflammatory breast cancer; IGF = insulin-like growth factor; IGFBP-rP = insulin-like growth factor-binding protein related protein; LABC = locally advanced breast cancer; LI = labeling index; PgR = progesterone receptor; VEGF = vascular endothelial growth factor.

pathologic findings coincide, this is not always the case. There are patients with the typical clinical syndrome in whom skin biopsy fails to demonstrate lymphatic tumor emboli. In some instances, the skin changes may represent acute mastitis, diffuse leukemic or lymphomatous infiltrate, or advanced noninflammatory breast cancer. On the other hand, lymphatic tumor emboli in the dermis are not always found in patients with IBC, mainly due to sampling heterogeneity. Importantly, tumor emboli in the dermal lymphatics can be observed incidentally in breast cancer, without any clinical evidence of IBC; such cases are not considered to be IBC, despite the pathologic findings.

The term 'primary IBC' refers to a *de novo* IBC. All patients with primary IBC have an underlying invasive mammary carcinoma, which may or may not be evident clinically or on mammogram at the time of diagnosis. 'Secondary IBC' is the inflammatory recurrence of a noninflammatory primary breast carcinoma. Secondary IBC usually occurs on the chest wall at the site of previous mastectomy for a noninflammatory breast cancer, but occasionally may be found at a distant cutaneous recurrence. The term 'occult IBC' describes a group of patients who have cutaneous and parenchymal lymphatic tumor emboli that are associated with their primary tumor in the absence of skin erythema. Because the diagnosis of IBC should be made on the basis of clinical features, the term 'occult IBC' is confusing and should be abandoned.

Clinical and pathologic features of invasive breast cancer

IBC represents 1–6% of all breast cancers [4]. The age distribution is not significantly different from that of ordinary infiltrating carcinoma, averaging around 55 years, with most patients being postmenopausal [4].

The typical patient presents with pain and a tender, firm, and enlarged breast. The skin over the breast is reddened, warm, and thickened, and is termed '*peau d'orange*' (skin of an orange). In the earliest phase a mass may not be palpable. These signs and symptoms are characteristically rapidly progressive, with a median duration before diagnosis of less than 2 months.

Pathologically, IBC is highly angiogenic and angioinvasive. Although it is not a specific histologic subtype of mammary carcinoma, the presence of numerous ectatic and dilated dermal lymphatics clogged by malignant cells constitutes the histologic hallmark that may accompany the symptoms. Malignant cells invade the dermal lymphatic vessels and form tumor emboli, which are responsible for the clinical signs and symptoms, and ultimately for the development of metastases [5].

Primary IBC is often of ductal type, with prominent angiolymphatic invasion. It has a high histologic grade, with pleomorphic tumor cells and highly atypical mitotic figures. In contrast to secondary IBC, in primary IBC invasion of the dermis outside the lymphatic vessels is uncommon. Interestingly, the skin within and outside the zone of erythema appear histologically identical, with tumor emboli frequently present in areas that are clinically unremarkable [5]. It has been suggested [6] that primary breast carcinomas with prominent apocrine features are associated with increased incidence of inflammatory recurrences.

Prognosis and treatment

IBC is a major challenge to oncologists who treat breast cancer. Until the introduction of combined modality treatment, less than 5% of patients survived 5 years [6]. IBC presents two therapeutic challenges. The first one is to target its high metastatic potential and prevent distant metastatic failures, which may be present even before detection of the primary tumor. To achieve this goal prolonged neoadjuvant systemic therapy is advocated. The second challenge is to achieve local/regional disease control by using radiation, surgery, and chemotherapy. For these reasons, a multimodality treatment approach is currently used to treat patients with IBC.

Haagensen and Stout [7] emphasized the poor outlook for this group of breast cancer patients, as they were considered inoperable. Improved radiation techniques have increased the chances of achieving local control [8]. Radiotherapy alone yielded 5-year survivals of 12–38% in different series [9]. Combination of surgery and radiotherapy has resulted in 5-year survivals of 35–55% in some studies [10].

The hypothesis that virtually all patients with IBC have disseminated micrometastatic disease at presentation and the discouraging patient outcome urged the use of chemotherapy as the first-line of treatment (neoadjuvant), and after surgery and/or radiation as a pivotal tool in the treatment of patients with IBC. In a retrospective study from the Harvard-Joint Center for Radiation Therapy, USA [9], 41 patients with stage III breast cancer, who received post-irradiation adjuvant therapy with either chemotherapy alone or combined with an endocrine-ablative procedure, had a significantly better disease-free survival at 4 years (51%). The Milan group [11] was the first to introduce the use of combination chemotherapy before local therapy. In 110 patients, they achieved an overall disease-free survival of 53% at 3 years, compared with an overall disease-free survival of 41% for a control group that was treated with radiation therapy alone. A similar treatment strategy was used by Hortobagyi *et al* [12] at the MD Anderson Hospital.

The largest series of patients with IBC was reported by Rouesse *et al* [13], who studied 230 patients who were subjected either to radiation treatment alone (group C); to three cycles of neoadjuvant chemotherapy, one cycle during radiation and one cycle after radiation (group A); or to three

cycles of neoadjuvant chemotherapy, one cycle during radiation, followed by five cycles of chemotherapy (group B). All patients had hormonal treatment as well. There were significant differences in the 4-year disease-free survival and overall survival rates, which were 16 and 28% for patients in group C, 28 and 44% for patients in group A, and 46 and 66% for patients in group B, respectively. Taken together, these studies led to the conclusion that neoadjuvant chemotherapy resulted in considerable tumor regression that enhanced the ability either to perform a surgical resection or to deliver a tumoricidal dose of radiation.

The concept of prolonged neoadjuvant chemotherapy treatment to the point of maximal objective clinical response emerged in the mid-1970s. Swain *et al* [14] treated 76 patients with stage IIIA, IIIB, and IV breast cancers. All patients received a variable number of neoadjuvant chemotherapy cycles until the maximum objective clinical response was attained. This group obtained complete remission in 49% and partial remission in 44% of patients. The median numbers of cycles of chemotherapy in this study were three for partial responders and five for complete responders.

At the University of Michigan, we studied a group of stage III breast cancer patients ($n = 89$), which included 36 (40%) patients with IBC [15]. These patients were treated with neoadjuvant chemohormonal treatment followed by local/regional therapy with radiotherapy alone or surgery plus radiotherapy, and finally with consolidation chemohormonal treatment. Clinical complete remission was achieved in 53% and clinical partial remission in 41% of IBC patients. Pathologic complete and partial responses were seen in 25 and 75% of patients, respectively. An encouraging 94% response rate (clinical complete response plus clinical partial response) to chemotherapy was achieved, with an overall survival rate of 54% at 5 years. Despite these initial responses, however, the estimated 5-year disease-free survival and overall survival rates were 35 and 37%, respectively. The total response rate for patients with IBC in our study was comparable to rates observed by other groups [16,17].

In summary, the multidisciplinary approach to the treatment of IBC with neoadjuvant chemotherapy to maximal clinical response, followed by local/regional treatment and consolidation chemotherapy, has improved survival and disease-free survival, and constitutes the mainstream of current treatment for patients with IBC. Below, we discuss work that has been undertaken to improve our understanding of the molecular basis of the IBC phenotype.

Molecular genetics of inflammatory breast cancer

Although the histomorphologic features of IBC have been well described in numerous publications (for review [18]), the molecular basis of the disease has only been investigated in a handful of studies. The vast majority of these investigations focused on the hormone receptor status and, to a lesser extent, on the *p53* tumor suppressor status of patient samples, seeking to establish a correlation between expression of these genes and the poor clinical outcome.

Hormone receptor status

In general, the absence of estrogen receptor (ER) and progesterone receptor (PgR) expression has been correlated with a shorter disease-free survival time and overall poor clinical outcome of breast cancer patients, including those with IBC [19]. Studies from 1981 to 1990 have described the ER and PgR status of patient IBC samples and their relationship to prognosis [20]. Using the dextran-coated charcoal technique to determine the levels of the cytosolic receptors [21], this work revealed that IBC tumors were predominately ER- and PgR-negative, with 26–100% of the samples being negative for both receptors. Furthermore, the levels of expression of these receptors alone had no prognostic significance for IBC.

In a study of a heterogeneous collection of 60 LABC tumor specimens [22], seven biologic features of the tumors were analyzed with respect to prognosis. It was found that response to treatment, and therefore a more favorable prognosis, was significantly correlated with ER and PgR positivity, a lower mitotic index, histologic grade, and diploid tumors in patients with LABC.

In an attempt to identify unique biologic characteristics that could be used as prognostic markers, Paradiso *et al* [23] compared the hormone receptor status and cell kinetics of IBC versus other LABC tumors. That study examined ER, PgR, and ^{3}H-thymidine labeling index (LI) of 28 IBC and 50 non-IBC tumors. Compared with non-IBC LABC, IBC tumors frequently lacked expression of the cytosolic ER (44% of IBC tumors were ER positive versus 64% of LABC) and PgR (30% of IBC tumors were PgR positive versus 51% of LABC), regardless of menopausal status. However, that study demonstrated that IBC cell kinetics were typically twofold higher than non-IBC tumors, and that the mean LI of IBC tumors was 1.5-fold greater in premenopausal women than in postmenopausal women. An apparent relationship between hormonal receptor status and LI was also noted. IBC tumors that were ER negative tended to have twice the LI of the ER-positive IBC tumors. Although the time to progression was similar for IBC tumors with either low or high LI, those patients whose tumors had a lower LI and were PgR positive survived longer (31 months) than did those whose tumors were PgR negative with a high LI (18 months). These results suggest that ER- and PgR-negative status and high LI cell kinetics may be useful markers for identification of patients who have a poorer clinical outcome manifested as decreased overall survival.

In order to investigate the relationship between ER expression and the expression of the proto-oncogene c-*myb* in breast cancer, an analysis of 112 non-IBC specimens and 57 IBC specimens was conducted [24]. Expression of the *ER* and *PgR* genes, c-*myb*, c-*erb2*, c-*myc*, c-*fos*, the epidermal growth factor receptor (*EGFR*) gene, and *pS2* (a small secreted protein isolated from MCF7 cells after induction by 17β-estradiol) were analyzed in that study. The IBC specimens were found to be predominately positive for the EGFR gene (58%) and c-*erb2* (60%). Expression of c-*myb* was found to correlate inversely with c-*erb2* expression, and was higher in non-IBC samples (63% versus 38%). Expression of the other genes was approximately the same for non-IBC and IBC specimens, and no statistically significant differences were found.

Taken together, all of these studies have established that the majority of IBC tumors are ER and PgR negative, EGFR and c-*erb2* positive, and have a rapid growth rate. Although nonspecific for IBC, these molecular markers may serve as general prognostic markers.

The *p53* tumor suppressor gene in inflammatory breast cancer

Mutations in the *p53* tumor suppressor gene are the most common mutations found in human cancers [25,26]. Wild-type p53 can act as a negative regulator of cell proliferation through induction of cell cycle arrest or through the induction of the cell's apoptotic machinery [27]. Mutations at the *p53* locus constitute the most common mechanism to abrogate the regulatory effects of p53.

This is certainly the case with colorectal carcinoma, in which over 70% of cases have a mutation in *p53* [28]. Studies have indicated that on average 30% of breast cancers contain mutations in one of the *p53* alleles [29]. Furthermore, it has been demonstrated that, on progression, approximately 60% of these breast cancers lose the mutant allele and retain the wild-type allele [30]. A study by Moll *et al* [31] described two mechanisms that alter *p53* function in a panel of 27 IBC cases. Using immunohistochemistry, they found that the cases fell almost equally into three distinct groups: tumors with high levels of p53 detected in the nucleus, tumors with no detectable p53 staining, and tumors that stained for p53 in the cytoplasm. Direct sequencing demonstrated only wild-type p53 in the samples with cytoplasmic p53 staining or no detectable p53 staining. The samples that contained intense nuclear staining for p53 contained a variety of missense mutations that were clustered in exons 5–8. These results suggest that two distinct mechanisms, direct mutation or cytoplasmic sequestration of the wild-type protein, can subvert the normal role of p53 in IBC and in other breast cancers.

In an attempt to correlate *p53* status with prognosis, the analysis was extended by an additional group of 24 IBC patients, who were screened for *p53* mutations, *p53* mRNA levels, and protein expression [32]. Using multivariate analysis, a positive correlation with *p53* nuclear expression and poor clinical outcome was observed. Those patients with a *p53* gene mutation and nuclear over-expression of the p53 protein had an 8.6-fold higher risk of death compared with patients who had neither mutation nor protein over-expression. Furthermore, when combined with ER data, it was found that the subset of patients who were ER negative and had nuclear over-expression of *p53* had a 17.9-fold higher risk of death, compared with 2.8-fold for those women who had *p53* nuclear over-expression alone. Together, the data from this group implicate *p53* expression status as a strong indicator of overall survival, alone and in combination with other known prognostic markers.

These conclusions were confirmed in a study of 39 LABCs with inflammatory signs [33], 32 of which were confirmed as IBCs by clinical and pathologic criteria. Using a combination of immunohistochemistry, polymerase chain reaction, single-strand conformational polymorphism, and direct sequencing, *p53* mutations were detected in 41% of the tumors analyzed. Again, the majority of mutations were localized to exons 5–8, the mutational hot-spot for *p53* found in most cancers. In addition, all but three of the tumors had intense *p53* nuclear staining. The presence of *p53* mutations was significantly associated with large tumor size and the presence of overt metastasis at the time of diagnosis. A weak association between *p53* mutation, negative ER status, and lower response rate to therapy was also apparent.

These data give a prognostic value to determining the ER and *p53* status of individual patients in order to determine who may fail or have only a minor response to neoadjuvant therapy. The above work showed that known markers of poor outcome in general also contributed to poor outcome in IBC. However, this work did not suggest how the specific features of the IBC phenotype might result from these genetic alterations.

Genetic determinants of the inflammatory breast cancer phenotype

In an effort to identify new genes that may determine the rapidly growing and metastatic features of the IBC phenotype, our laboratory began a study of genes that are differentially expressed in an IBC cell line (SUM149) as compared with human mammary epithelial (HME) cells [34]. Seventeen transcripts were identified as being differentially expressed between the SUM149 IBC cell line and two HME cell lines of the same mammary differentiation lineage. Eight of the transcripts were found to be expressed solely by the normal cell lines and not by the tumor. The remaining nine transcripts were expressed exclusively by the tumor cell line. In an analysis that was

blind to the IBC or non-IBC status of the samples, 20 archival IBC and 30 stage-matched non-IBC specimens were tested by *in situ* hybridization for expression of the 17 differentially expressed transcripts. In an example of basic research stemming from a clinical study, the samples used in the *in situ* hybridization experiments were collected over the course of almost 10 years in a prospective, nonrandomized clinical trial at the University of Michigan [15]. Two genes, *RhoC GTPase* and a novel gene, were found to be concordantly altered in 91% of the IBC specimens versus 0% of the stage-matched non-IBC tumors. The putative oncogene, *RhoC GTPase*, was found to be over-expressed in 90% of the IBC tumors analyzed, compared with 38% of the non-IBC specimens. Expression of the novel gene, called *LIBC* (lost in inflammatory breast cancer), was found to be lost in 80% of IBC specimens versus in only 21% of noninflammatory tumors. The protein encoded by *LIBC* has subsequently been identified as an insulin-like growth factor (IGF)-binding protein related protein (IGFBP-rP), and officially re-named IGFBP-rP9 [35].

The *Rho* (Ras homology) gene was first isolated from *Aplysia*, and has been shown to be highly conserved throughout evolution [36]. Transfection of *Aplysia Rho* (which is 92% homologous to human RhoC) into NIH3T3 cells led to malignant transformation of the cells [37]. RhoC GTPase is involved in cytoskeletal reorganization; specifically, it is involved in the formation of actin stress fibers and focal adhesion contacts [38]. In addition to increased stress fiber and focal adhesion contact formation, over-expression of RhoC could activate other *Rho* family members (ie RhoA, rac and/or cdc42) through signaling feedback loops [39], leading to dynamic cytoskeletal reorganization and a motile/metastatic cell.

Preliminary data from our laboratory indicate that this is probably the case. Transfection and over-expression of RhoC GTPase in Epstein–Barr virus-immortalized HME cells produce highly motile and invasive tumorigenic clones (van Golen *et al*, unpublished data). These data, combined with the observation that over-expression of RhoC GTPase correlates with tumor progression in aggressive ductal adenocarcinoma of the pancreas [40], suggest that RhoC is a key element in the IBC metastatic phenotype.

At this time, the role of the protein encoded by *LIBC* (IGFBP-rP9) in IBC is less clear. The functions of IGFBP-rPs are at this time unknown. It is speculated the high-affinity IGF-binding proteins and the IGFBP-rPs modulate the availability of IGF to the cell, and therefore regulate the availability of IGF to the IGF receptors [41]. This, in turn regulates and potentiates IGF-mediated proliferative and anabolic effects on the cell. In addition, these proteins may also exert IGF-independent effects on a cell [42].

There is evidence in the literature for the role of IGFBP-rPs in tumor progression. Downregulation or loss of IGFBP-rP1 expression is associated with progression of breast cancer [43] and prostate cancer [44]. Transfection of IGFBP-rP1 in prostate cancer cells gives rise to a less malignant phenotype, in a manner that is directly proportional to the level of expression [44]. Together, RhoC GTPase and *LIBC* provide promising new avenues of study that could lead to new prognostic tools and therapeutic targets for IBC.

Cytokines in inflammatory breast cancer

As already mentioned, the term 'IBC' is somewhat of a misnomer. The 'inflammation' of the breast, which prompted Lee and Tannenbaum [1] to coin the phrase 'inflammatory breast cancer', is actually caused by blockage of the dermal lymphatics by tumor infiltrate, and not by infiltration of inflammatory cells. IBC tumors produce negligible levels of most inflammatory cytokines, including IFN-γ, IL-1, and IL-12 (van Golen and Merajver, unpublished data); consequently, host inflammatory cells are rarely detected around the tumor stroma [5].

In addition to being lymphotactic, IBCs tend to be highly vascular tumors due to their angiogenic and angioinvasive potential [5]. Analysis of IBC tumor cell lines and archival tumor specimens demonstrated that high levels of vascular endothelial growth factor (VEGF), basic fibroblast growth factor, IL-6, and IL-8 are expressed and secreted by the tumor cells (unpublished data).

The VEGF receptor-3 is found on the epithelium of lymphatic vessels and is known to bind the recently identified VEGF family members VEGF-C and VEGF-D [45]. A variety of breast cancer cell lines were screened for expression levels of the VEGF family members (VEGF-A, -B, -C, and -D) [46]. VEGF-A and -B, which are important in regulating tumor angiogenesis, were found to be expressed by all of the breast cancers analyzed, regardless of type. However, VEGF-D was detected only in an IBC cell line and in a tumor cell line that was developed from an inflammatory skin metastasis.

These data suggest that the expression of VEGF-A and -B might be responsible for neovascularization of the tumor, whereas VEGF-D may be specifically involved in the lymphotactic process through the development of new lymphatic vessels near the tumor. The work of understanding the specific mediators of IBC is at its inception. The models described below will be helpful in this pursuit.

In vitro and *in vivo* models to study inflammatory breast cancer

Recently new models have been developed to study IBC. Cell lines, such as the SUM149 and SUM190, which are derived from primary IBC tumors, will be valuable for *in*

vitro studies of the IBC phenotype [47]. These cell lines have been well characterized with respect to expression of hormonal receptors, *p53* status, and cytogenetic abnormalities. In addition, several less well-characterized cell lines derived from confirmed IBCs are available from the American Type Culture Collection. Both the SUM149 and SUM190 cell lines are known to form tumors in nude mice after mammary fat pad injection with large uptake rates (80–100%).

A unique xenograft model, MARY-X, has been established [48]. Implantation of a human IBC tumor into the mammary fat pad of an immunocompromised mouse has established a tumor that accurately reflects some clinical symptoms of IBC in a mouse model. The establishment of these *in vivo* models will facilitate the characterization of IBC phenotype–genotype relationships.

Conclusion

The challenges before us are, on one hand, to discover novel treatment modalities that target essential steps that are involved in the development of IBC, such as those directed against its angiogenic and angioinvasive potential. On the other hand, new molecular predictors of tumor response to treatment need to be identified. Achieving these challenges is likely to result in improved patient survival.

Initial molecular studies of IBC concentrated on those genes and gene products that were identified as having prognostic value in noninflammatory breast cancers. These studies provided a basis for understanding the role of these genes in IBC. Moreover, these studies contributed two important conclusions. First, although some genetic changes in IBC are shared with other forms of breast cancer, for the most part it is as distinct in its molecular profile as it is in its phenotype. Second, this collection of studies comparing IBC with other forms of LABC have led the way in recognizing IBC as a disease that is distinct from other LABCs. To further characterize the genotype–phenotype relationship of IBC, breast cancer researchers will need to recognize that IBC is a distinct form of LABC. Recent interest in the IBC phenotype has led to the development of new IBC cell lines such as SUM149 and SUM190 [47,49], and to a unique xenograft model, MARY-X [48]. These models will help in investigating the molecular differences, not only between the cancer and normal tissue, but also between inflammatory and noninflammatory disease.

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