# Chapter 3
# The Role of Inflammation in Inflammatory Breast Cancer

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**Abstract** Inflammatory breast cancer (IBC) is the most aggressive form of breast cancer. Despite extensive study, whether inflammation contributes to the tumorigenicity or aggressiveness of IBC remains largely unknown. In this chapter, we will review the potential role played by inflammation in IBC based on the results of in vitro, in vivo, and patient studies. Current evidence suggests that several major inflammatory signaling pathways are constitutively active in IBC and breast cancer. Among them, the NF-κB, COX-2, and JAK/STAT signaling systems seem to play a major role in the tumorigenesis of IBC. Inflammatory molecules such as interleukin-6, tumor necrosis factor alpha (TNF-α), and gamma interferon have been shown to contribute to malignant transformation in preclinical studies of IBC, while transforming growth factor-β, interleukins 8 and 1β, as well as TNF-α appear to play a role in proliferation, survival, epithelial–mesenchymal transition, invasion, and metastasis. In this chapter, we also describe work thus far involving inhibitors of inflammation in the development of prevention and treatment strategies for IBC.

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## 3.1 Introduction

Breast cancer is the second most common cancer, following skin cancer, among women in America. The American Cancer Society estimates that there will be 232,670 new cases of breast cancer among women in the United States in 2014 (ACS 2014). It is also the second leading cause of death from cancer in women, approximately with 40,000 predicted deaths for 2014. Inflammatory breast cancer (IBC) is the most aggressive form of breast cancer. Although it accounts for an estimated incidence rate of up to 5 % of breast cancers (Anderson et al. 2005; Hance et al. 2005; Jaiyesimi et al. 1992), IBC is responsible for a disproportionate 8–10 % of all breast cancer-related deaths (Hance et al. 2005).

The word "inflammatory" was first applied to the IBC subtype of breast cancer by Haagensen (1971). His description was based on certain presenting features that are unique to this subgroup of patients. IBC presents with rapid onset of breast erythema occupying at least one-third of the breast, accompanied by breast edema leading to the characteristic peau d'orange appearance of the skin. Other features include breast enlargement, pain, and tenderness. Approximately 50 % of patients do not present with a palpable mass or radiographic evidence of cancer (Ueno et al. 1997; Yang et al. 2008). Almost all IBC patients present with lymph node metastasis at the time of diagnosis, and approximately 30 % present with distant metastasis (Jaiyesimi et al. 1992; Li et al. 2011).

For diagnosing IBC, consensus guidelines recommend at a minimum a core biopsy to enable detection of invasive carcinoma and to allow marker study (hormone receptors and HER2). A skin punch biopsy to confirm the presence of dermal lymphatic invasion, one of the hallmarks of IBC, is also strongly recommended in suspected cases (Dawood et al. 2011).

Treatment of IBC, as for other types of breast cancer, involves a multidisciplinary approach that includes surgery, radiation therapy, and medical oncology. Patients are stratified according to extent of disease and the molecular subtype. This approach has been associated with a significant reduction in cancer-related mortality (Ueno et al. 1997; Kesson et al. 2012). Currently, the most active anti-cancer agents include anthracycline and taxanes, in addition to anti-HER2 therapy and endocrine therapy. However, compared with other types of breast cancer, treating IBC has proved to be more challenging mainly because of its rapidly aggressive nature combined with the lack of effective targeting therapy.

Despite extensive study, whether inflammation contributes to the tumorigenicity or aggressiveness of IBC remains unknown. In this chapter, we will review the potential role played by inflammation in IBC based on the results of in vitro, in vivo, and patient studies. We will also describe work thus far involving inhibitors of inflammation in the prevention and treatment of IBC.

## 3.2  Inflammatory Signaling Pathways Associated with IBC

Several intrinsic pathways driven by oncogenes or tumor suppressor genes have been shown to activate the expression of inflammation-related programs in both IBC and breast cancer in general. These pathways are described below.

**NF-κB**  The nuclear factor of kappaB (NF-κB) family of sequence-specific transcription, known to play a critical role in inflammation and the innate immune response, has recently been implicated in tumorigenesis. It is ubiquitously expressed in all cell types, where, in most cases, it is maintained in an inactive state in the cytoplasm bound to a class of inhibitory proteins known as IκBs (inhibitors of κB). Activation of NF-κB occurs by a variety of stimuli and is regulated in normal cells via two main pathways, the classical (canonical) pathway and the alternative (non-canonical) pathway (Prasad et al. 2010). Both pathways involve kinase-dependent degradation of inhibitory molecules to release NF-κB, but they differ in the inhibitory molecule involved, the activated kinases, and the types of NF-κB proteins as well as the stimuli that trigger them. Upon activation, NF-κB is transported to the nucleus, where it upregulates the expression of target genes that are responsible for a wide variety of effects, including the inflammatory and immune response, proliferation, cell–matrix adhesion, chemotaxis, and angiogenesis (Shostak and Chariot 2011).

In a variety of cancers, including breast cancer, NF-κB undergoes persistent (constitutive) activation (Nakshatri et al. 1997). Laere et al. (2005) performed a genome-wide expression profile using a cDNA microarray to compare IBC and non-IBC tissue samples. The authors reported that an unusually high number of NF-κB target genes were differentially overexpressed in IBC versus non-IBC. In a similar study, the mRNA expression levels of 60 NF-κB-related genes were compared in IBC versus non-IBC samples using real-time quantitative RT-PCR. The authors reported that approximately 60 % of NF-κB-related genes were upregulated in the IBC samples compared to non-IBC samples. The resulting five-gene molecular signature was matched with patient outcomes; it included two genes that are regulated by NF-κB (Lerebours et al. 2008). Collectively, these studies confirm the importance of NF-κB in IBC and its contribution to the aggressive phenotype of IBC.

In a recent study, El-Shinawi et al. (2013) examined the association between evidence of human cytomegalovirus (HCMV) infection in the serum and tissue of IBC and non-IBC patients and whether HCMV was associated with NF-κB activation in IBC. The authors reported significantly higher levels of serum HCMV IgG as well as higher levels of HCMV DNA in the tumor tissue of IBC patients. Infected IBC samples also had enhanced NF-κB/p65 signaling compared to non-IBC controls (El-Shinawi et al. 2013). While these findings suggest an association between IBC and HCMV could exist, the authors noted that the evidence is not conclusive. If the results are confirmed, they may help explain the higher incidence of IBC in some geographic areas (Soliman et al. 2011).

Several studies have suggested complex cross talk between NF-κB and estrogen receptor (ER) in IBC as well as in breast cancer in general. In a separate study, Van Laere et al. reported that NF-κB activation in IBC tumors was associated with ER downregulation, which was linked to both EGFR and/or HER2 overexpression and MAPK hyperactivation (Laere et al. 2007). Additionally, ER seems to be capable of inhibiting both the constitutive and the inducible activation of NF-κB in a dose-dependent manner (Biswas et al. 2004). On the other hand, studies also seem to suggest that in ER-positive patients, cross talk between ER and NF-κB occurs that may either be transrepressive or positive (Kalaitzidis and Gilmore 2005). Theoretically, this could explain how in luminal B subtypes and some ER-positive IBC patients, resistance to hormonal therapy and poorer outcome could result from positive cross talk between NF-κB and ER leading to enhanced ER-mediated expression of genes involved in cell proliferation, survival, and resistance.

**COX family** The cyclooxygenase (COX) family of enzymes consists of two members, COX-1 and COX-2. Both enzymes catalyze the conversion of arachidonic acid to prostanoids and are also responsible for the generation of eicosanoid products, which are important mediators of pain and inflammation. Tissue upregulation of COX-2 can be triggered by several stimuli, including growth factors and oncogenes (Williams et al. 1999). Aberrant activation of COX/prostaglandin signaling is common in many cancers, especially in colon cancer, where COX-2 is overexpressed in 85 % of tumors (Brown and DuBois 2005). This has also been the case with breast cancer; enzyme levels have been found to be increased in 40 % of breast tumor tissues examined (Yoshimura et al. 2003).

Several studies have documented the role that the PTGS2 gene, which encodes COX-2, plays in cell proliferation, invasion, angiogenesis, and metastasis (Wang and Dubois 2004; Menter et al. 2010). Overexpression of COX-2 in breast cancer correlates with a more aggressive breast cancer profile that is characterized by higher proliferation rates, larger tumors, higher pathologic grade, hormone receptor negativity, and HER2 overexpression (Ristimaki et al. 2002; Subbaramaiah et al. 2002). Compared with non-IBC tumors, the PTGS2 gene is differentially overexpressed in IBC, and it is identified as a key component in the molecular signature for IBC (Laere et al. 2005, 2007). Moreover, prostaglandin E2 (PGE2), which is a product of COX-2 enzymatic activity, is known to be upregulated in primary IBC tumors and metastatic lesions (Robertson et al. 2008). These findings emphasize the role of the COX-2 pathway in IBC and its potential use as a target for disease prevention and treatment.

**JAK/STAT signaling** The JAK/STAT signaling system is the main pathway for a variety of cytokines, including interferon and interleukins (e.g. IL-6), as well as growth factors or other chemical messengers. Depending on both the context and the integrity of the pathway, JAK/STAT signaling can stimulate proliferation and cell migration versus differentiation and apoptosis (Rawlings et al. 2004).

STAT3 is known to be constitutively activated in >50 % of breast cancers and tumor-derived cell lines (Garcia et al. 1997; Diaz et al. 2006). Using small interfering RNA (siRNA) to block STAT3 in both cell culture and xenograft models of breast cancer, investigators were able to show increased apoptosis through the Fas-mediated intrinsic apoptotic pathway, as well as reduced expression of the transmembrane molecule B-cell lymphoma-extra large (Bcl-xL), which promotes

survival (Kunigal et al. 2009). Additionally, constitutive activation of STAT led to accelerated mammary tumorigenesis and increased metastatic potential in cancer cells expressing ErbB2 (Barbieri et al. 2010). Moreover, ablation of STAT3 resulted in inhibition of anchorage-independent growth of breast cancer cells, thus limiting their metastatic potential. More recently, however, it was found that JAK2/STAT3 appears to be necessary for growth and survival of tumor cells expressing the cancer stem cell (CSC) phenotype (Ma et al. 2011). More recently, investigators were able to induce cell death in SUM149 IBC tumor spheres by inhibiting STAT3 activation in a dose-dependent manner using a novel JAK2 inhibitor (Ma et al. 2011).

In summary, current evidence suggests that several major inflammatory signaling pathways are constitutively active in IBC and breast cancer. Among them, the NF-κB, COX-2, and JAK/STAT signaling systems seem to play a major role in the tumorigenesis of IBC. Blocking these pathways may prove to be a promising therapeutic strategy owing to their multiple roles in promoting cancer cell survival and metastasis.

## 3.3  Role of Inflammatory Molecules in the Development of IBC: Evidence From In Vitro Studies

### 3.3.1  Role of Inflammatory Molecules in Malignant Transformation

Accumulating DNA mutations play a causal role in the process of malignant transformation. Oncogenic insults may result in activation of oncogenes, loss of tumor suppressor genes, or the constitutive activation of membrane receptors or lead to the alteration of critical cellular processes such as the cell cycle or apoptosis (Hanahan and Weinberg 2000). In this setting, inflammation may contribute to carcinogenesis through the activation of the DNA damage response system in response to major oncogenic insults (Martin et al. 2011; Hartman et al. 2011). Moreover, viral infections, such as human papillomavirus (HPV), whose genome has been detected in breast cancer tissue, may also cause DNA damage, resulting in activation of the DNA damage response pathway, and stimulate the formation of a pro-inflammatory tumor microenvironment (Moody and Laimins 2009; Kan et al. 2005).

Interleukin-6 (IL-6), which is overexpressed in the SUM149 preclinical model of IBC (Golen et al. 2000), plays a potent role in malignant transformation. IL-6 was able to convert a non-transformed mammary epithelial cell line (MCF-10A) to the transformed state in 24–36 h (Iliopoulos et al. 2009, 2010).

Additionally, HER2 overexpression, which is known to occur in up to 60 % of IBC tumors and 25 % of non-IBC tumors, is associated with poor outcome (Guerin et al. 1989; Kallioniemi et al. 1991). HER2 amplification was associated with marked increase in IL-6 in breast cancer cells and induced STAT3 activation, suggesting a HER2-IL-6-STAT3 signaling pathway could play a critical role in tumorigenesis (Hartman et al. 2011).

On the other hand, cancer stem cells (CSCs), also known as tumor-initiating cells, are highly tumorigenic cells and are enriched in IBC tumors as well as in preclinical models of IBC (Laere et al. 2010; Charafe-Jauffret et al. 2010; Xiao et al. 2008). IL-6 acts as a growth factor for CSCs and is sufficient to convert non-stem cancer cells to CSCs (Iliopoulos et al. 2011). IL-6 gene expression was found to promote self-renewal, as well as invasive potential, in both normal and MCF-7-derived spheroids (Sansone et al. 2007). IL-6 is also at the center of epigenetic regulation of stem cells (D'Anello et al. 2010; Hodge et al. 2005). IL-6 thus plays a critical role in mediating the epigenetic switch that involves NF-κB and STAT3 and links inflammation to cell transformation (Iliopoulos et al. 2009, 2010).

Additional inflammatory signaling involved in the regulation of CSCs includes the tumor necrosis factor alpha (TNF-α) and gamma interferon (IFN-γ) pathways, both of which are upregulated in breast CSCs (Murohashi et al. 2010). Both cytokines are also able to activate the NF-κB pathway (Cheshire and Baldwin 1997; Hayden and Ghosh 2008; Matsumoto et al. 2005). Treatment with the chemokine IL-8 resulted in increased mammosphere formation, whereas IL-8 receptor/CXCR1 blockade depleted the breast CSC population both in vitro and in xenografts (Murohashi et al. 2010; Charafe-Jauffret et al. 2009; Ginestier et al. 2010). Expression of CCL5/RANTES was also found to be upregulated in breast CSC populations (Murohashi et al. 2010).

### 3.3.2  Role of Inflammatory Molecules in the Survival of IBC Cells

One of the hallmarks of cancer cells is their capacity to acquire resistance to apoptotic signals (Hanahan and Weinberg 2000). Transforming growth factor (TGF)-β is a pro-apoptotic cytokine that normally induces cell cycle arrest in the early phases of tumorigenesis. The mechanisms by which cancer cells escape the inhibitory effects of TGF-β are not fully understood but may include inactivating mutations or homozygous deletions(Kaklamani et al. 2003; Pasche et al. 2004; Dunning et al. 2003) or upregulation in oncogenic expression (Zhang et al. 2003). Once the pro-apoptotic functions of TGF-β are subverted, its tumorigenic potential becomes unhindered, thus stimulating growth, invasion, and angiogenesis (Biswas et al. 2007; Lei et al. 2002).

Likewise, although TNF-α promotes apoptosis in MCF-7 cells (Simstein et al. 2003), a process similar to TGF-β subverts the pro-apoptotic effect of TNF-α (Rivas et al. 2008). HER2 amplification, which is present in up to 60 % of IBC patients, was shown to confer resistance to TNF-α-induced apoptosis in breast cancer cell lines mainly through an Akt/NF-κB anti-apoptotic cascade (Zhou et al. 2000). Likewise, increased expression of claudin-1 was able to reverse TNF-α-induced apoptosis in the MCF-7 breast cancer cell line (Liu et al. 2012). These studies suggest that there are multiple pathways by which breast cancer cells can overcome TNF-α-induced apoptosis, thus promoting cancer cell survival and unleashing the tumorigenic potential of TNF-α.

Using mastectomy samples from patients with either invasive or non-invasive breast cancer as well as tissue from benign controls, both IL-6 protein levels and IL-6 receptor levels, were correlated with tissue levels of B-cell lymphoma 2 (Bcl-2) and Bcl-2-associated X (Bax) proteins (Garcia-Tunon et al. 2005). A higher proportion of malignant samples, compared with benign controls, were positive for IL-6, Bcl-2, and Bax by immunohistochemistry. The more invasive samples had a more intense immunoreaction for Bcl-2 than did benign lesions. In addition, infiltrating tumors that were positive for IL-6 were also positive for Bcl-2 with a high degree of correlation between immunoreaction intensities of both antibodies (Garcia-Tunon et al. 2005). These results, along with others, suggest that IL-6 plays a central role in protecting cancer cells against apoptosis as well as the regulation of survival in CSCs via several pathways, including the canonical JAK/STAT3 pathway, and by direct action on Bcl-2 family gene products (Iliopoulos et al. 2011; Hinohara and Gotoh 2010; Heinrich et al. 2003).

### 3.3.3  Role of Inflammatory Molecules in the Proliferation of IBC Cells

Cancer is fundamentally a disease of inappropriate cell division and proliferation. Cytokines can enhance growth through their interaction with growth factors, e.g., ER, and transcriptional pathways such as IL-6/JAK/STAT3. An in vitro study comparing ER-positive to ER-negative breast cancer cell lines reported higher levels of IL-6-mediated STAT3 phosphorylation in ER-negative versus ER-positive cells. Upon exposure of MCF-7 ER-positive cells to IL-6, tumor cell growth rates were enhanced by >two-fold (Sasser et al. 2007).

IL-1β is a major proinflammatory cytokine that is known to contribute to tumor proliferation, angiogenesis, and local invasion (Apte et al. 2006). Higher IL-1β levels in breast cancer tissue or serum were correlated with more aggressive disease and poorer outcome (Goldberg and Schwertfeger 2010). However, the interaction between IL-1β and ER in tumor growth in breast cancer has been less understood with evidence supporting transcriptional activation of ER by IL-1β (Speirs et al. 1999). A recent study suggested that IL-1 secretion in breast cancer may be regulated by estradiol in vivo and that its release may be inhibited by antiestrogen therapy (Abrahamsson et al. 2012).

TNF-α, a potent suppressor of proliferation in normal cells, was found to enhance proliferation in the T47D breast cancer cell line through an NF-κB-dependent pathway (Rubio et al. 2006). Proliferating cells were found to express high levels of cyclin D1 (Rivas et al. 2008). Furthermore, the addition of a specific NF-κB inhibitor, Bay 11-7082, was able to block TNF-α-induced tumor promotion and cyclin D1 expression. Additional in vitro studies on MCF-7 cells demonstrated the capacity of TNF-α to upregulate several genes associated with cancer proliferation (Yin et al. 2009). Alternatively, TNF-α can interact with ER as well as other transcription factors in an NF-κB-independent manner to regulate genes that are important for proliferation in breast cancer (Gloire et al. 2006).

### 3.3.4  Role of Inflammatory Molecules in the Invasion, Metastasis, and Angiogenesis of IBC Cells

Cancer cell progression is a multistep process that involves the acquisition of several characteristics that include epithelial–mesenchymal transition (EMT), cell invasion, migration, intra- and extravasation, and angiogenesis. EMT is the process by which cancer cells lose epithelial properties such as cell polarity and cell-to-cell contact and acquire mesenchymal (fibroblastic) characteristics. This process confers malignant properties such as invasiveness and motility and is essential for cancer cells to metastasize (Thiery 2002; Thiery et al. 2009).

IBC gene expression profiles have revealed the activation of specific stem-cell-related pathways that contribute to the activation of NF-κB, which in turn induces EMT (Laere et al. 2010). Recently, investigators were able to reproduce EMT in IBC cells using a three-dimensional culture system. IBC cells exhibited a reduction in epithelial markers (E-cadherin) and overexpression of mesenchymal marker vimentin. Investigators were able to inhibit EMT by blocking the EGFR pathway using an EGFR tyrosine kinase inhibitor, erlotinib (Zhang et al. 2009).

Overexpression of TGF-β has been associated with several tumors and correlates with aggressive features (Derynck et al. 2001). TGF-β plays a central role in the induction of EMT (Moustakas and Heldin 2007). It inhibits expression of E-cadherin (Xu et al. 2009) and is associated with reduced levels of claudins and occludins, as well as tight-junction degradation (Moustakas and Heldin 2007). Moreover, TGF-β1 induces expression of Mdm2, which results in the destabilization of p53, a critical step in the EMT of breast cancer that is associated with advanced disease (Araki et al. 2010).

In tissue samples of human breast cancers, high levels of TGF-β1 mRNA were associated with increased angiogenesis as measured by microvessel density, features that are common in IBC (de Jong et al. 1998). TGF-β is known to trigger the expression of vascular endothelial growth factor (VEGF) as well as act as a chemo-attractant for monocytes, which in turn release angiogenic factors (Yang and Moses 1990; Ashcroft 1999). Furthermore, TGF-β is also able to induce cell migration through the expression of matrix metalloproteases MMP-2 and MMP-9 (Hagedorn et al. 2001). The results of these studies suggest that TGF-β exerts a broad range of effects that confer invasiveness and metastasis through its regulation of EMT and cell motility (Docherty et al. 2006; Yang et al. 2006).

Higher levels of IL-6 in the SUM149 IBC model have been attributed to regulation by RhoC GTPase, which plays a role in the development of the invasive/angiogenic phenotype of IBC (Golen et al. 2000). In turn, IL-6 activates multiple effectors involved in the process of invasion and metastasis (Heinrich et al. 2003; Tawara et al. 2011). Sullivan et al. (2009) observed that IL-6 induced EMT as well as enhanced invasiveness of MCF-7 cancer cells. Furthermore, IL-6 produced by fibroblasts or stromal adipocytes derived from breast tissue or from metastatic sites promoted invasion in MCF-7 cells (Studebaker et al. 2008; Walter et al. 2009).

The chemokine IL-8 is another inflammatory molecule that is differentially expressed in IBC tumors (Laere et al. 2005, 2007). IL-8 production is amplified in metastatic breast cancer lesions and plays a key role in tumor progression, invasion, and angiogenesis (Freund et al. 2004; Yao et al. 2007; Lin et al. 2004). A similar effect can be seen with increased tumor and serum levels of IL-1β, which were associated with invasiveness in ER-negative breast tumors (Goldberg and Schwertfeger 2010). In ER-positive tumors, IL-1β was found to promote EMT changes as well as cell migration, invasion, angiogenesis, and metastasis (Franco-Barraza et al. 2010; Wang et al. 2005).

Studies in MCF-7 breast cancer cells have also shown TNF-α to promote the expression of a panel of genes that are known to be associated with invasion and metastasis (Yin et al. 2009). Moreover, the chemokine receptor CXCR4 and its ligand CXCL12 (stromal cell-derived factor-1 alpha) are differentially expressed in IBC tumors and are known to regulate interactions between tumor cells and the microenvironment that are critical for the development of organ-specific metastasis (Cabioglu et al. 2007; Clezardin 2011).

## 3.4  Role of Inflammatory Molecules in the Development of IBC: Evidence From In Vivo Studies

One of the challenges facing IBC research is the development of preclinical models that accurately recapitulate the aggressiveness of the disease. Currently, there is a need for better immunocompetent mouse models of IBC that allow assessment of the molecular and inflammatory mechanisms underlying the disease and the development of effective therapeutic targets.

In a recent study that looked at the role of NF-κB signaling in conferring self-renewal to breast cancer cells, three types of IBC SUM149 cells were prepared and injected into the mammary fat pads of nude mice. These included cells expressing IκBα-SR at low or high density or an empty vector (Kendellen et al. 2013). Investigators assessed self-renewal by measuring the ability of cells when injected at limiting dilutions to establish primary tumors. Cells with deficient NF-κB signaling produced smaller tumors at a much later onset compared to those with empty vector, whereas the low density of SUM149 cells expressing IκBα-SR did not form tumors. The ability to self-renew appears to require both intact canonical and non-canonical NF-κB pathways (Kendellen et al. 2013). This demonstrates the importance of NF-κB for tumorigenesis in xenograft models.

Cyclooxygenase-2 (COX-2) is over-expressed in mammary tumors derived from rodent models of breast cancer. Enhanced COX-2 expression was found to be sufficient to induce mammary gland tumorigenesis in the mouse mammary tumor virus (MMTV)/COX-2 transgenic mouse strain, thus providing evidence for its in vivo oncogenicity (Liu et al. 2001). Additionally, mammary gland involution after weaning was delayed in the transgenic animals compared to controls, suggesting that apoptosis suppression was also involved (Liu et al. 2001).

Studies have also demonstrated that tumor formation in these models can be suppressed either pharmacologically by using anti-inflammatory drugs, including COX inhibitors, or through genetic ablation (Howe 2007; Howe et al. 2001; Howe et al. 2005). COX inhibitors were also evaluated in HER2/neu transgenic mice, which are also ER negative. Celecoxib administration was able to significantly delay tumor formation in the animal model (Howe et al. 2002; Lanza-Jacoby et al. 2003).

To examine the consequences of knocking out COX-2, investigators adopted an approach used in intestinal cancer models (Oshima et al. 1996), by crossing COX-2 knockout mice with mammary tumor virus/neu deletion mutant (MMTV/NDL) mice and comparing tumor multiplicity to HER2/neu transgenic mice that were COX-2 wild type, heterozygous, and null (Howe et al. 2005). Tumor multiplicity and size were significantly reduced in COX-2 knockout mice (heterozygous and null) compared to controls (Howe et al. 2005). Additionally, the authors observed that COX-2 null animals were associated with reduced expression in several angiogenesis factors, which led to a reduction in mammary blood vessel formation. Together, these studies suggest that an intact COX-2 pathway is both necessary and sufficient for the induction of tumorigenesis.

Similarly, the tumorigenicity of TGF-β was assessed by developing a doxycycline-inducible triple transgenic mice model in which doxycycline can be used to induce TGF-β1 expression in polyomavirus middle T antigen (PyVmT) transformed mammary tumors (Muraoka-Cook et al. 2004). TGF-β1 stimulation resulted in rapidly accelerated metastatic progression with >ten-fold increase in lung metastases in as little as 2 weeks. Antisense-mediated inhibition of TGF-β1 resulted in decreased cell motility, survival, anchorage-independent growth, tumorigenicity, and metastasis. Similarly, Criswell et al. looked at the role of TGF-β type III receptors in inducing EMT, cancer cell motility, and invasion of metastatic cancer cells through a similar transgenic model (Criswell et al. 2008).

To address the role of IL-6 in cancer proliferation, investigators looked at whether expression of IL-6 in MCF-7 cells would alter tumor growth rates in immunocompromised mice. Xenografts expressing IL-6 underwent rapid engraftment and expansion relative to MCF-7 xenografts that did not express IL-6 (Sasser et al. 2007). On the other hand, using siRNA to knock down STAT3 expression in nude mice, investigators were able to suppress breast cancer cell growth compared with controls. pRNAi-STAT3 also led to downregulation of STAT3 and Bcl-xL, as well as upregulation of Fas and induction of apoptosis via expression of cleaved caspase-3 (Kunigal et al. 2009; Matthews et al. 2007).

## 3.5 Evidence From Patients for the Role of Inflammation in IBC

The rarity of IBC as a disease has not allowed the role of inflammation to be systematically examined in clinical studies involving IBC patients; however, numerous clinical reports and observational studies have addressed the role of inflammation in breast cancer in general.

C-reactive protein (CRP) is an acute-phase protein that is considered the classic marker of systemic inflammation. CRP levels in plasma are known to rise rapidly in response to acute inflammation (Black et al. 2004; Casas et al. 2008; Gabay and Kushner 1999), but have also been found to be moderately increased in chronic inflammatory disease (Hirschfield and Pepys 2003). Large epidemiologic studies have suggested a correlation between high circulating levels of CRP and the risk of developing cancer. This observation has not been demonstrated for breast cancer, however. The Women's Health Study measured baseline plasma CRP levels for 27,919 healthy women aged 45 years or older. After a mean follow-up of 10 years, 892 patients had developed breast cancer; results showed no association between increased CRP levels and the risk of developing breast cancer (Zhang et al. 2007).

Likewise, in a Danish general population study, 10,408 individuals had their CRP levels measured at baseline and were observed for up to 16 years. During follow-up, 1,624 went on to develop cancer, and 998 patients died. Increased CRP levels were associated with an increased risk of cancer of any type and possibly an increased risk of colorectal cancer and lung cancer, but not breast cancer (Allin et al. 2009).

On the other hand, high CRP levels were found to be associated with poor prognosis in several types of cancer, including breast cancer. Allin et al. (2011) looked at CRP levels at baseline in 2,910 breast cancer patients. Higher CRP levels were found to be associated with larger tumor size, development of distant metastases, and poor prognosis. More importantly, the authors reported that breast cancer was the leading cause of death in this cohort, thus excluding the possibility that the outcome was confounded by risk of cardiac disease, for which CRP is an established risk factor (Allin et al. 2011).

Ristimaki and colleagues analyzed the expression of COX-2 protein using immuno-histochemistry in tissue specimens of 1,576 patients with breast cancer (Ristimaki et al. 2002). Increased levels were found in approximately 40 % of breast tumors and were associated with shorter distant metastasis-free survival. Tumors with COX-2 expression were associated with negative hormone receptor status as well as the presence of HER2 amplification and axillary nodal metastasis. Additional unfavorable features associated with COX-2 increase include larger tumor size, higher histological grade, high Ki-67 proliferation rates, higher p53 expression, and ductal type histology. However, the differences in outcome between patients with increased COX-2 protein and those without was even more pronounced in patients with more favorable prognostic characteristics, such as ER positivity, low p53 expression, and no HER-2 amplification.

The preclinical model of IBC, a disease known for its aggressive course, expressed increased levels of IL-6 and IL-8 (Golen et al. 2000). High levels of IL-6 were reported to be associated with poorer response to therapy in patients with metastatic breast cancer (Zhang and Adachi 1999). This was confirmed in the clinical setting; breast cancer patients were found to have higher serum levels of IL-6 than do healthy women (Kozlowski et al. 2003; Jiang et al. 2000). Two studies looked at IL-6 in different tumor stages and found higher levels of IL-6 were correlated to advanced cancer stage (Jablonska et al. 2001; Lyons et al. 2011). Others looked at how serum levels correlated with recurrence and outcome in the metastatic setting (Nishimura et al. 2000; Bozcuk et al. 2004; Salgado et al. 2003). One study analyzed the association between IL-6 serum levels and response

to therapy as designated by the Response Evaluation Criteria in Solid Tumors (RECIST); higher levels were associated with poor objective response to therapy (Zhang and Adachi 1999).

Similarly, TGF-β levels in plasma in breast cancer patients were found to be increased and predictive of lymph node and distant metastasis (Ivanovic et al. 2009; Yu et al. 2010). Additionally, increased levels of IL-1β in the tumor and in the serum of ER-negative breast tumors were correlated with tumor invasiveness and poor outcome (Studebaker et al. 2008). The production of IL-8 in ER-positive breast cancer patients was associated with shorter relapse-free survival (Freund et al. 2003). Furthermore, increased circulating levels of TNF-α were correlated with increased lymph node metastasis and breast cancer stage (Sheen-Chen et al. 1997).

## 3.6  Inhibitors of Inflammation for the Prevention and Treatment of IBC

Management of IBC consists of tri-modality therapy: neoadjuvant chemotherapy, then modified radical mastectomy, followed by locoregional radiotherapy. Prior to the era of multimodality therapy, the 5-year overall survival rate was less than 5 % (Robbins et al. 1974). In a more recent study, patients who received all components of tri-modality therapy achieved an overall survival rate at 5 years of 51 %, versus 24 % for patients who did not receive all three components (Bristol et al. 2008).

One of the biggest challenges in the treatment of IBC thus far has been the lack of clinically relevant treatment targets. In a retrospective analysis, 316 IBC patients were assigned according to ER and HER2 status into four groups: ER positive (33 %), ER positive/HER2 positive (12 %), HER2 positive (26 %), and triple negative (29 %) (Li et al. 2011). The triple-negative subtype was found to predict the worst overall survival and high recurrence rates. Hence, the search for potential treatment targets has become a priority in particular for patients with triple-negative IBC. One promising tactic has been to target the inflammatory pathways in the adjuvant setting or in combination with systemic therapy (Pierga et al. 2010; Agrawal and Fentiman 2008).

Pan et al. looked at the activity of tetrathiomolybdate, a copper chelator, in tumors derived from SUM149 IBC cells. Tetrathiomolybdate was shown to effectively suppress angiogenesis and motility in IBC cell line tumors through its inhibitory effects on NF-κB signaling (Pan et al. 2002, 2003). This was accompanied by reduced levels of VEGF, basic fibroblast growth factor (bFGF), IL-6, IL-1α, and IL-8, as well as decreased tumor volume (Pan et al. 2002). Another compound that is known to inhibit the NF-κB pathway is pyrrolidinedithiocarbamate (Zhou et al. 2008). Inhibition of the NF-κB pathway, which is upregulated in IBC, is one of the most promising areas of research.

The chemokine CXCR4/CXCL12 receptor/ligand pair has been observed to promote angiogenesis as well as confer survival on CSCs (Duda et al. 2011; Greenfield et al. 2010). The CXCR4 antagonist, CTCE 9908, in combination with paclitaxel, was evaluated in a SUM149 preclinical model of triple-negative IBC

(Singh et al. 2010). CTCE-9908 as a single agent inhibited skeletal metastases but failed to prevent primary tumor growth or pulmonary metastasis.

Owing to their unacceptable cardiotoxicity, the use of selective COX-2 inhibitors has been limited despite initial enthusiasm regarding promising epidemiologic results and their anti-cancer activities (Psaty and Furberg 2005; Graham et al.2005). Interest has instead shifted to searching for alternative COX-2-targeted agents. One such target is the family of prostanoid receptors, particularly EP4, that bind with PGE2, which is a product of COX-2 (Jones et al. 2009). EP4 was found to mediate invasion and metastasis in both inflammatory and non-IBCs, and EP3 suppressed angiogenesis in IBC tumors (Robertson et al. 2008, 2010). None of the available EP4 antagonists have yet been tested in cancer patients.

Apricoxib is a novel selective COX inhibitor analog that is currently under evaluation in breast cancer in combination with lapatinib and capecitabine in the treatment of HER2/neu-positive advanced breast cancer (Health NIo 2001). Tranilast is another compound under investigation and is known as a potent inhibitor of PGE2. It was shown to suppress tumorigenesis in both xenograft mammary tumors and human triple-negative breast cancer cells (Chakrabarti et al. 2009; Subramaniam et al. 2010, 2011).

Another agent under evaluation in breast cancer is fish oil. Fish oils contain the omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which ultimately lead to the inhibition of inflammation in the body (Weaver et al. 2009). This is suspected to occur through inhibition of COX/PGE2 production (Wendel and Heller 2009).

Recently, a new role for statins as a preventive agent against cancer has emerged. Recent evidence has suggested that in addition to their lipid-lowering effects, statins exert powerful anti-inflammatory effects by acting on multiple inflammatory gene pathways (Jain and Ridker 2005). The anti-cancer effects of statins have also been linked to the mevalonate pathway, which in turn leads to the inhibition of many downstream growth factors (Nielsen et al. 2012). Large observational studies have pointed toward the potential role this pathway has against cancer in general as well as breast cancer specifically (Nielsen et al. 2012; Ahern et al. 2011). Brewer et al. have also addressed the potential role of statins in improving the survival of patients with IBC (Brewer et al. 2012).

Curcumin is the principal derivative of turmeric, the popular Indian spice and a member of the ginger family. Various preclinical studies have looked into its role in breast cancer as a chemosensitizer and radiosensitizer to agents such as doxorubicin, 5-fluorouracil, and paclitaxel (Goel and Aggarwal 2010). Curcumin is a known potent inhibitor of NF-κB, STAT3, and COX-2 as well as other growth factors and anti-apoptotic proteins (Goel and Aggarwal 2010).

## 3.7  Conclusions and Future Directions

Current evidence supports that inflammation plays a central role in the process of tumor formation in IBC at various levels. Several important inflammatory gene pathways are differentially upregulated in IBC and contribute to the formation

of a pro-inflammatory feedback loop that is critical for malignant transformation (Hartman et al. 2011). On the other hand, downstream cytokines and chemokines are involved at every step of tumorigenesis/carcinogenesis, including initiation, transformation, proliferation, cancer cell survival, invasion, angiogenesis, and metastasis. Several pharmacological compounds that target the inflammatory signaling pathways are currently being tested in the laboratory and in the clinical setting. There is a demand for better immunocompetent IBC mouse models for more accurate in vivo testing and drug development. Proteomic analysis of IBC offers the opportunity to conduct a quantitative and functional evaluation of protein activity in the various signaling networks involved (Chen et al. 2002). It allows assessment of posttranslational modifications, complementing gene expression studies in IBC (Bichsel et al. 2001). New approaches such as high-throughput screening may help identify novel agents that inhibit key signaling pathways. Ultimately, the clinical role of targeting inflammation in IBC needs to be tested prospectively.

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