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Interleukin-6 in the Breast Tumor Microenvironment

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1. Introduction

Greater than 200,000 new cases of breast cancer cases were diagnosed in 2010 in the United States, with approximately 40,000 women succumbing to the disease (www.cancer.gov). Globally, an estimated 1.38 million new cases of breast cancer were diagnosed in 2008, with greater than 450,000 women succumbing to the disease (Jemal *et al.*, 2011). Despite our improved understanding of breast carcinogenesis, breast cancer remains the second most commonly diagnosed cancer in women behind non-melanoma skin cancer and the second leading cause of death in women behind lung cancer. These epidemiological statistics highlight the overwhelming clinical dilemma of breast cancer and emphasize the need for novel therapeutic targets and prevention strategies. Countless studies in the fields of mammary gland development and breast cancer have led to an appreciation of a breast tumor microenvironment that actively contributes to the heterogeneous nature of breast cancer. The current review will focus on the impact of IL-6 and STAT3 activation in the breast tumor microenvironment and subsequently present rationale for targeting the IL-6/STAT3 signaling pathway in this setting. IL-6 is a quintessential pleiotropic cytokine produced by a diverse number of cell populations, most of which can localize to the breast tumor microenvironment. Excessive IL-6 has been demonstrated in primary breast tumors and breast cancer patient sera and is associated with poor clinical outcomes in breast cancer. These clinical associations are corroborated by emerging preclinical data revealing that IL-6 is a potent growth factor and promotes an epithelial-mesenchymal (EMT) phenotype in breast cancer cells to indicate that IL-6 in the breast tumor microenvironment is clinically relevant. Numerous clinical reports have now demonstrated the safety and efficacy of IL-6 signaling antagonists in multiple diseases, which supports future investigations of these therapies in breast cancer.

Estrogen receptor-alpha (ERα) is a latent cytoplasmic ligand-activated transcription factor utilized by clinicians to subclassify the heterogeneous disease of breast cancer. ERα-positive breast cancer incidence increases up to age 51, the mean age of menopause, and continues to increase until age 80. Conversely, ERα-negative breast cancer incidence plateaus and even slightly decreases at age 51, while demonstrating an increase prior to age 50 comparable to that of ERα-positive disease. This discrepancy between the two incidence rates at menopause produces an inflection in the incidence rate of all breast cancer cases which has been termed Clemmesen's hook (Anderson and Matsuno, 2006). Whereas the prevalence of

ERα-positive cells within terminal duct lobular units of the breast of healthy premenopausal women has been reported at 7%, this number is estimated at 42% in postmenopausal women (Shoker *et al.*, 1999). In addition, approximately two-thirds of all breast cancers are diagnosed as ERα-positive, and 75% of postmenopausal breast cancers are ERα-positive (Macedo *et al.*, 2009). Progesterone receptor (PR) and epidermal growth factor receptor 2 (EGFR2; HER2; or ErbB2), a receptor tyrosine kinase involved in cellular proliferation, have also acquired much clinical attention following reports of dismal survival rates in "triple negative" (ERα-negative/PR-negative/HER2 not overexpressed) breast cancer patients. Triple negative breast cancer represents approximately 15 to 20% of all breast cancer cases and can only be treated with standard chemotherapy as it lacks current adjuvant therapeutic targets. Such breast tumors are highly proliferative with a high mitotic index, increased necrosis, elevated apoptosis, and typically are of higher tumor grade. *TP53* gene and p53 protein mutations as well as loss of the Rb tumor suppressor protein are common. Familial breast cancer patients with congenital BRCA1 mutations often present with triple negative breast cancer, as do relatively younger breast cancer patients and African American women. Currently, triple negative breast cancers are associated with a poor prognosis largely due to poor survival rates and early relapse. The fact that these breast tumors respond well if not completely to initial chemotherapy may seem counterintuitive, but enhanced invasiveness, consequent distant metastasis, and residual local recurrence eventually promote poor survival rates (Irvin and Carey, 2008).

Breast cancer most commonly metastasizes to bone, followed by lung, liver, and brain. Perhaps due to the heterogeneity across individual breast cancer cases, few prognostic molecular biomarkers have been demonstrated to accurately predict metastatic potential. One of the most important of these biomarkers is ERα, which is clinically exploited as a predictor of bone metastasis (Kominsky and Davidson, 2006). Whereas ERα-positive breast cancers have a strong tendency to metastasize to bone if at all (James *et al.*, 2003), their ERα-negative counterparts favor visceral organs such as lung and liver (Hess *et al.*, 2003). Primary mammary tumor cell dissemination has been quantified at 3 to 4 x 10^6 primary tumor cells in circulation per 24 hours per gram of tumor in a rat mammary carcinoma model, which exemplifies the inefficient nature of metastasis (Butler and Gullino, 1975). Although metastasis has been generally accepted as a relatively late event throughout cancer progression, recent work has revealed evidence of early primary tumor cell dissemination, thus refuting this paradigm (Klein, 2009). In particular, it has now been demonstrated that untransformed triple transgenic (doxycycline-inducible K-ras, MYC, and polyoma middle T antigen) mammary epithelial cells are capable of lung colonization when tail vein-injected into immunocompromised female mice on doxycycline. This work showed that untransformed "normal" mammary epithelial cells can colonize ectopic lung tissue, and upon oncogene activation, disseminated mammary epithelial cells within circulation or a foreign host microenvironment are capable of forming tumors at the ectopic site (Podsypanina *et al.*, 2008). Additionally, reports of bone marrow cytokeratin-positive epithelial cells in up to 48% of breast cancer patients without overt metastases also offer support for early primary tumor cell dissemination. Decreased survival in patients with such cells was demonstrated in all studies (Braun *et al.*, 2000; Diel *et al.*, 1996; Gebauer *et al.*, 2001; Pantel *et al.*, 2003; Vannucchi *et al.*, 1998). Furthermore, only 8% of these patients with bone marrow micrometastases exhibited cytokeratin-positive/Ki67-positive cells, suggesting that lack of overt bone metastasis may be due to disseminated tumor cell dormancy (Pantel *et al.*, 2003).

2. The breast tumor microenvironment

A normal epithelial tissue can undergo hyperplasia and acquire tumorigenic properties that promote the development of a benign, non-invasive solid tumor known as carcinoma *in situ*. Normal epithelial tissues and non-invasive carcinoma *in situ* tumors are separated from a supportive stromal compartment by an intact basement membrane. Ultimately, carcinoma *in situ* can progress to a malignant, invasive carcinoma, the most common form of human cancer. The panoply of published investigations between the fields of mammary gland development and breast cancer has led to an appreciation for a supportive non-epithelial mammary stroma that mechanically and biologically restrains tumorigenesis. However, tumors of the breast and other epithelial tissues obviously overcome these growth restraints and exploit this stroma to sculpt a vastly divergent tumor stroma. Tumor stroma is generally divided into four main components: tumor vasculature, inflammatory leukocytes, extracellular matrix (ECM) and soluble growth factors, and fibroblasts. Malignant carcinoma cells and tumor stromal cells bi-directionally communicate with one another through paracrine signaling and intercellular contacts in a disorganized ECM to constitute a tumor microenvironment. Tumor-associated fibroblasts (TAF), the predominant stromal cell population within the tumor microenvironment, acquire and sustain an "activated" phenotype that promotes tumor progression (Rasanen and Vaheri, 2010). TAF are capable of enhancing breast tumor growth and metastasis by means of promoting angiogenesis (Orimo *et al.*, 2005), epithelial-mesenchymal transition (EMT) (Martin *et al.*, 2010; Radisky *et al.*, 2005), and progressive genetic instability (Kurose *et al.*, 2001; Moinfar *et al.*, 2000). In contrast, a normal mammary microenvironment can act in a dominant manner to inhibit tumor growth and "revert" the malignant phenotype of breast cancer cells (Kenny and Bissell, 2003). While resident breast tissue fibroblasts can inhabit breast tumors as TAF, breast tumors also recruit distant cell populations that engraft within the breast tumor microenvironment where they actively contribute as TAF. For example, mesenchymal stem cells (MSC), a bone marrow-derived stromal cell population, home to breast cancer cell xenograft tumors and persist as TAF (Spaeth *et al.*, 2009).

3. Cancer-associated inflammation

Although highly characterized for their protective capacity against infection, inflammatory leukocytes also reside within the tumor microenvironment. In fact, various immune cells are capable of eliminating transformed cells and thus preventing tumorigenesis in a process termed immunosurveillance (Dunn *et al.*, 2004). Whereas acute inflammation may prevent tumorigenesis by promoting an immune response directed against transformed cells, chronic inflammation promotes tumorigenesis. Rudolf Virchow is credited with making the seminal link between chronic inflammation and cancer by noting that human tumor biopsies were often infiltrated with inflammatory cells (Balkwill and Mantovani, 2001). Leukocytes can be detected in non-malignant tumors and carcinomas, including breast cancer (DeNardo and Coussens, 2007), which suggests an ongoing antitumor immune response. Despite the infiltration of leukocytes such as cytotoxic T-cells and NK-cells, the persistence of a tumor demonstrates immune evasion and highlights the local and systemic immune suppressive state of the tumor microenvironment and the tumor-bearing host, respectively.

4. Interleukin-6: A quintessential pleiotropic cytokine

Interleukin-6 (IL-6) is an inflammation-associated cytokine and major inducer of C-reactive protein (CRP) throughout the acute phase inflammatory response. *IL6* gene expression is nuclear factor-kappaB (NF-κB)-dependent (Chauhan *et al.*, 1996) and produces a 26 kDa IL-6 protein product. First characterized as a T-cell-derived factor that induced proliferation, differentiation, and immunoglobulin production in B-cells, IL-6 was originally named B-cell stimulating factor-2 (BSF-2). It was later thought to be a novel interferon (IFN-β_2) due to studies demonstrating the ability of IL-6 to activate signal transducer and activator of transcription 3 (STAT3) (Kishimoto, 2006). Complementary DNA encoding the human IL-6 gene was subsequently cloned, and human IL-6 transgenic mice demonstrated a polyclonal IgG1 plasmacytosis phenotype (Suematsu *et al.*, 1989). Next, IL-6 knockout (IL-6$^{-/-}$) mice were generated and characterized. IL-6$^{-/-}$ mice underwent normal development, but adult animals exhibited reduced numbers of peripheral T-cells and impaired antiviral cytotoxic T-cell activity (Kopf *et al.*, 1994). In addition, IL-6 is a critical factor during hematopoiesis and subsequent lymphocyte differentiation and activation. Multiple diverse cell populations including fibroblasts, T and B-cells, monocytes, macrophages, endothelial cells, keratinocytes, astrocytes, and smooth muscle cells all have the potential to produce constitutive or inducible IL-6 (Kishimoto, 2006).

Depending on cellular context, IL-6 can signal through multiple kinase-dependent proliferation and anti-apoptosis pathways including the mitogen-activated protein kinase (MAPK) pathway, the phosphatidylinositol-triphosphate kinase (PI-3K)/Akt pathway, and perhaps the most commonly evaluated in breast cancer, the Janus kinase (JAK)/signal transducer and activator of transcription-3 (STAT3) pathway (Hodge *et al.*, 2005). To do so, a plasma membrane-associated IL-6 receptor (IL-6R/CD126) homodimer first ligates two soluble IL-6 molecules, which leads to gp130 (CD130) homodimer ligation. Whereas IL-6R is only expressed on hepatocytes, osteoclasts, and most immune cells under normal physiological conditions, gp130 is a ubiquitous and promiscuous receptor involved in multiple cytokine signaling pathways (e.g., IL-11, leukemia inhibitory factor (LIF), oncostatin M (OSM), and ciliary neurotrophic factor (CNTF)) (Rose-John *et al.*, 2006). To initiate classical JAK/STAT3 signal transduction, JAK are recruited to the intracellular domain of the gp130 receptor where they bind and autophosphorylate. Subsequent gp130 phosphorylation via activated JAK offers docking sites for STAT3 and other receptor-associated proteins. Once bound to the intracellular domain of gp130, STAT3 is specifically phosphorylated (pSTAT3) by adjacent JAK on a C-terminal tyrosine residue (Y705), which grants its disengagement from the receptor. Dissociation of pSTAT3^{Y705} from gp130 facilitates its homodimerization within the cytoplasm, and the pSTAT3^{Y705} homodimer translocates to the nucleus. There, pSTAT3^{Y705} binds to specific promoters whereby it initiates the transcription of multiple downstream target genes (Clevenger, 2004). Under normal physiological conditions, an inhibitory feedback loop maintains rapid and transient STAT3 activation. Following activation in normal cells, STAT3 induces suppressors of cytokine signaling (SOCS) and protein inhibitors of activated STATs (PIAS) expression. While SOCS-1 specifically inhibits JAK function, SOCS-3 binds the IL-6R complex to inhibit IL-6 signal transduction. PIAS-3 directly interacts with STAT3 to inhibit all STAT3 target gene expression (Kishimoto, 2006). In contrast, many human cancers, including breast cancer, exhibit constitutive STAT3 activity. Recent studies have demonstrated that unphosphorylated STAT3 (U-STAT3) accumulates in tumor cells with constitutively active

STAT3 where it forms a complex with NF-κB to activate a subset of NF-κB target genes (Yang and Stark, 2008).

Alternatively, IL-6 *trans*-signaling describes an IL-6 signaling pathway whereby an IL-6 soluble receptor (IL-6sR) binds IL-6 and subsequently ligates gp130 to stimulate STAT3 activation in cells that only express gp130. IL-6sR is naturally produced by either proteolytic cleavage of the membrane-bound IL-6R or alternative splicing of IL-6R mRNA (Rose-John *et al.*, 2006). Whereas IL-6 serum levels continue to increase with age, levels of serum IL-6sR rise until approximately age 70 at which time they gradually decline (Giuliani *et al.*, 2001). Furthermore, IL-6sR expression has been demonstrated in human breast cancer cell lines (Crichton *et al.*, 1996; Oh *et al.*, 1996; Singh *et al.*, 1995), suggesting that IL-6 *trans*-signaling mediates the effects of IL-6 in breast cancer cells. In contrast, an endogenous soluble gp130 (sgp130) specifically antagonizes IL-6 *trans*-signaling by exclusively ligating the IL-6/IL-6sR complex, thus having no effect on cells that express the membrane-bound IL-6R (Rose-John *et al.*, 2006) (Figure 1).
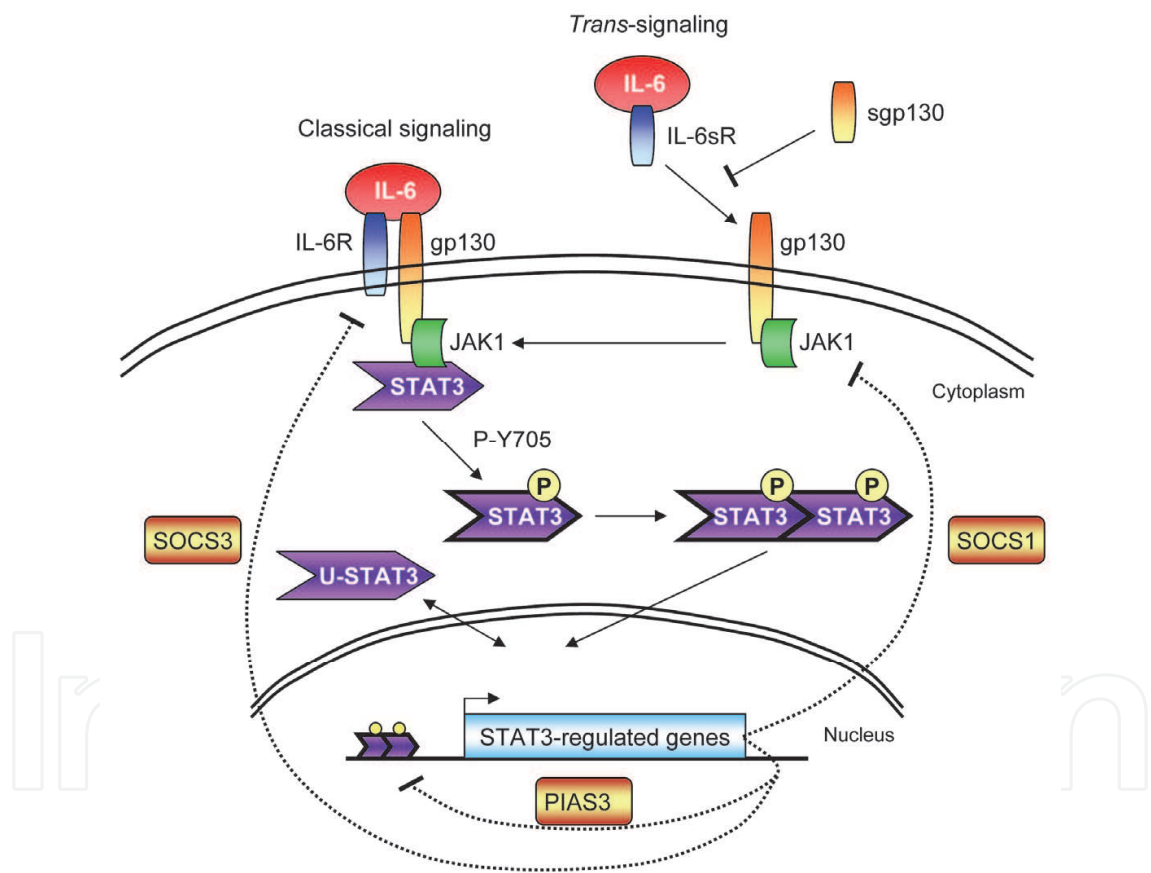

Fig. 1. The IL-6/STAT3 signaling pathway

5. Excessive IL-6 in human breast cancer

Aberrantly elevated IL-6 is associated with a poor prognosis in breast cancer (Bachelot *et al.*, 2003; Salgado *et al.*, 2003; Zhang and Adachi, 1999). Human breast tumors produce more IL-6 when compared to matched healthy breast tissue, and tumor IL-6 levels concurrently increase with tumor grade. In addition, increased serum IL-6 has been demonstrated in

breast cancer patients compared to normal donors and correlates with advanced breast tumor stage (Kozlowski *et al.*, 2003) and increased number of metastatic sites (Salgado *et al.*, 2003). Furthermore, a single nucleotide polymorphism (SNP) exists at position -174 in the IL-6 gene promoter region, noted as IL-6 (-174 G>C), with the following allele frequency in a Caucasion population: 36% G/G, 44% G/C, and 18% C/C. An inflammatory stimulus such as *Salmonella typhii* vaccination induced higher serum IL-6 in those individuals with the G/G allele (Bennermo *et al.*, 2004). Although the IL-6 (-174 G>C) SNP is not associated with increased risk of developing breast cancer (Gonzalez-Zuloeta Ladd *et al.*, 2006; Litovkin *et al.*, 2007; Yu *et al.*, 2009b), it is significantly associated with disease-free and overall survival in breast cancer patients (DeMichele *et al.*, 2003).

ERα is expressed in luminal subtype breast tumors (Perou *et al.*, 2000) and therefore associated with improved patient survival (Buyse *et al.*, 2006; Sorlie *et al.*, 2001). A clear and well-characterized inverse correlation exists between breast cancer ERα status and IL-6. In fact, ERα directly binds to NF-κB, thus preventing transactivation of *IL6* gene expression (Galien and Garcia, 1997), which demonstrates a direct mechanism for such a correlation. Furthermore, ERα-negative human breast tumors produce more IL-6 than tumors that express ERα (Chavey *et al.*, 2007), and IL-6 serum levels are higher in ERα-negative breast cancer patients compared to ERα-positive patients (Jiang *et al.*, 2000). Likewise, ERα-negative breast cancer cell lines produce autocrine IL-6 whereas ERα-positive breast cancer cell lines do not (Sasser *et al.*, 2007). Therefore, this strongly suggests that ERα-negative breast cancer cells would exploit both paracrine (i.e., stromal cell-derived) and autocrine IL-6 signaling, whereas ERα-positive breast cancer cells could only utilize paracrine IL-6 signaling. In addition, ERα-negative breast cancer patients, whose tumors produce more IL-6 than those that express ERα (Chavey *et al.*, 2007), showed no difference in survival between the G/G allele (higher inducible serum IL-6) and any C allele (lower inducible serum IL-6) at the IL-6 (-174 G>C) promoter SNP. In contrast, ERα-positive breast cancer patients with any C allele at the IL-6 (-174 G>C) promoter SNP demonstrated improved disease-free and overall survival compared to those with the G/G allele (DeMichele *et al.*, 2003).

6. IL-6 promotes breast cancer cell growth

Stromal fibroblasts isolated from multiple types of tumors (i.e., TAF) or cancers (i.e., CAF) are now appreciated as influential players in cancer progression and metastasis (Orimo and Weinberg, 2006). CAF derived from multiple cancer types, including murine mammary cancers, exhibit an activated, proinflammatory phenotype with increased IL-6 production (Erez *et al.*, 2010). Furthermore, work from our laboratory has demonstated that fibroblasts isolated from breast tissue and common sites of breast cancer metastasis such as bone and lung enhance the growth of breast cancer cells in an IL-6-dependent manner, and IL-6 is the major fibroblast-derived soluble factor that induced STAT3 activation in breast cancer cells (Sasser *et al.*, 2007; Studebaker *et al.*, 2008). MDA-MB-231 breast cancer cells are commonly utilized to model triple negative breast cancer and produce autocrine IL-6. MDA-MB-231 cells expressing a dominant negative isoform of gp130 lacked constitutively active STAT3 and exhibited impaired tumorigenicity in an orthotopic xenograft model (Selander *et al.*, 2004), thus suggesting that IL-6 may drive tumor progression in this model. In addition, STAT3 is estimated to be constitutively activated in more than half of primary breast cancers due to IL-6 signaling (Berishaj *et al.*, 2007).

Mesenchymal stem cells (MSC) are a bone marrow-derived fibroblast cell population that can be recruited to the breast tumor stroma, acquire a TAF phenotype, and produce high levels of IL-6. MSC enhance the growth of ERα-positive breast cancer cells, which do not express IL-6 or activated STAT3. In contrast, MSC have no effect on IL-6-producing ERα-negative breast cancer cells, which express constitutively activated STAT3. Moreover, ERα-positive breast cancer cells orthotopically co-injected with MSC or MSC conditioned medium and ERα-positive breast cancer cells that ectopically express IL-6 demonstrate enhanced xenograft tumor growth in the absence of exogenous 17β-estradiol (Sasser *et al.*, 2007). Similar differential growth enhancement was demonstrated *in vivo* with ERα-positive and ERα-negative breast cancer cells co-injected with MSC, which also promoted metastasis (Karnoub *et al.*, 2007). Interestingly, IL-6 has been reported to facilitate the recruitment of MSC to hypoxic breast tumor microenvironments (Rattigan *et al.*, 2010). Likewise, IL-6 secreted from breast cancer cells has been shown to contribute to a recently characterized phenomenon termed "self-seeding" in which aggressive circulating tumor cells engraft within their original xenograft tumor (Kim *et al.*, 2009). MSC have also been shown to mediate the self-renewal capacity of breast cancer stem cells, in part, through a reciprocal IL-6 loop (Liu *et al.*, 2010). Taken together, preceding evidence strongly suggests that IL-6 promotes breast cancer cell growth by activating STAT3, which culminates with the upregulation of proliferative oncogenes such as c-Myc and cyclin D1 and and growth factors such as IL-6, hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF) (Yu *et al.*, 2009a).

7. IL-6 promotes epithelial-mesenchymal transition in breast cancer cells

Normal polarized epithelial cells exhibit 'cobblestone' homophilic morphology and express E-cadherin, which is required for epithelial cell polarization, phenotype, and consequent homeostasis (Jeanes *et al.*, 2008). E-cadherin is a key prognostic molecular biomarker clinically utilized to predict the metastatic propensity of breast cancer. Whereas very few studies have failed to demonstrate E-cadherin as an independent prognostic biomarker in breast cancer patients (Lipponen *et al.*, 1994; Parker *et al.*, 2001), the overwhelming majority of relevant studies have revealed E-cadherin as one of the strongest predictors of patient survival. Specifically, impaired E-cadherin expression in human breast tumors correlates with enhanced invasiveness, metastatic potential (Oka *et al.*, 1993), and decreased breast cancer patient survival (Heimann and Hellman, 2000; Pedersen *et al.*, 2002). While appropriate E-cadherin function is essential to the maintenance of epithelial cell morphology, phenotype, and homeostasis, regulation of E-cadherin expression is of equal importance. *CDH1*, the gene that encodes E-cadherin, is located on human chromosome 16q22.1 (Rakha *et al.*, 2006) and is susceptible to inactivation by promoter hypermethylation, somatic mutation, or aberrant overexpression of repressive transcription factors including Twist, Snail, and Slug among others (Hirohashi, 1998). Likewise, E-cadherin loss of function can arise due to extracellular domain-specific proteolytic cleavage. Although uncommon, germline mutations of *CDH1* predispose individuals to hereditary diffuse gastric cancer (HDGC) syndrome, and a proportion of these patients present with other cancers, including breast cancer (Guilford, 1999).

E-cadherin was initially termed uvomorulin in mice and L-CAM in chicks following its discovery as a 120 kDa calcium-dependent trypsin-labile cell surface glycoprotein required for intercellular adhesion in mouse blastomeres (Hyafil *et al.*, 1981) and chick embryos

(Brackenbury *et al.*, 1981). It now represents the best studied member of the cadherin family of tissue-specific homophilic intercellular adhesion molecules. E-cadherin knockout studies have demonstrated early embryonic lethality due to impaired maintenance of epithelial polarity and failure to form an intact epithelium in E-cadherin$^{-/-}$ embryos (Larue *et al.*, 1994). E-cadherin is localized on the cell surface of epithelial cells, and each E-cadherin protein consists of an amino-terminal extracellular domain, a single-pass transmembrane segment, and a carboxy-terminal intracellular domain. Five calcium-binding repeated subunits comprise an extracellular domain that promotes homophilic interaction to ultimately form anti-parallel trans-E-cadherin dimers between adjacent cells (Guilford, 1999). The intracellular domain is comprised of a juxtamembrane p120-catenin binding subdomain and a C-terminal beta (β)-catenin binding subdomain. β-catenin, a potent transcription factor, binds E-cadherin and alpha (α)-catenin subsequently binds β-catenin. Although contentious (Weis and Nelson, 2006), it is generally acknowledged that α-catenin interacts with F-actin and thereby, facilitates the linkage of E-cadherin to the cytoskeleton. This E-cadherin-catenin-actin complex localizes to epithelial intercellular junctions called adherens junctions and is critical to epithelial cell adhesion, polarity, and morphology (Hartsock and Nelson, 2008). Furthermore, E-cadherin sequesters β-catenin at the cell surface as one means to inhibit β-catenin nuclear translocation and consequent expression of β-catenin responsive genes (Perez-Moreno *et al.*, 2003).

Another prominent role of E-cadherin is that of an invasion/metastasis suppressor protein. Upon loss of E-cadherin and subsequent dissociation of adherens junctions, epithelial cells acquire enhanced invasive capability (Behrens *et al.*, 1989). MDA-MB-231 cells, an ERα-negative breast cancer cell line, lack E-cadherin, whereas MCF-7 cells, an ERα-positive breast cancer cell line express high levels of E-cadherin (Kenny *et al.*, 2007), and MDA-MB-231 cells exhibit enhanced invasive capability compared to MCF-7 cells (Sommers *et al.*, 1991). Naturally, E-cadherin expression and consequent invasive capacity regulate the propensity of breast cancer metastasis. Multiple signaling pathways are activated following loss of E-cadherin protein, which promote transformed human breast epithelial cell metastasis in a xenograft model. Interestingly, Twist, a transcriptional repressor of *CDH1*, is induced upon loss of E-cadherin and is necessary for metastasis in this model. Furthermore, the E-cadherin binding partner, β-catenin, was shown to be necessary but not sufficient for the EMT phenotype induced following loss of E-cadherin (Onder *et al.*, 2008). Ectopic expression of murine E-cadherin in highly metastatic human MDA-MB-231 cells significantly reduced osteolytic bone metastases in a murine intracardiac dissemination model (Mbalaviele *et al.*, 1996). Likewise, aberrant cytoplasmic or diminished to negative E-cadherin immunostaining patterns are commonly detected in invasive poorly differentiated breast carcinomas compared to noninvasive well-differentiated breast carcinomas and are associated with increased probability of breast carcinoma metastasis (Oka *et al.*, 1993). The finding that distant metastases often express E-cadherin even in patients which exhibit primary breast carinomas which lack E-cadherin suggests that ultimate re-expression may be necessary for colonization of secondary tissues (Kowalski *et al.*, 2003; Saha *et al.*, 2007).

Loss of E-cadherin is a prerequisite for epithelial-mesenchymal transition (EMT), a highly conserved process which exemplifies the aberrant activation of an embryonic gene expression program during carcinoma progression. EMT is critical for multiple steps of developmental metazoan cellular morphogenesis as demonstrated in well-characterized

Drosophila and *Xenopus* models. Throughout embryonic development, EMT whereby epithelial cells give rise to more motile mesenchymal cells is essential for mesoderm and neural crest formation. Importantly, this is a transient process and mesenchymal-epithelial transition (MET) allows for cellular reversion (Yang and Weinberg, 2008).

Whereas EMT has been extensively studied for its essential role in embryogenesis, the concept of EMT-like cellular changes in human cancers has gained acceptance as a major mechanism to promote primary tumor cell invasion and subsequent tumor metastasis. A carcinoma cell must first detach from the primary tumor and invade through the basement membrane into the underlying tissue parenchyma to initiate the metastasic cascade. Although cancer-associated EMT was considered a controversial notion even in recent years (Tarin *et al.*, 2005), it has been demonstrated in multiple human carcinomas, including breast cancer (Cheng *et al.*, 2008; Heimann and Hellman, 2000; Moody *et al.*, 2005; Sarrio *et al.*, 2008), and is now recongnized as a putative mediator of tumor metastasis. An EMT phenotype including impaired E-cadherin expression with concominant induction of Vimentin, Alpha-smooth-muscle-actin, and/or N-cadherin is associated with the basal breast cancer subtype, suggesting that EMT may promote characteristic aggressiveness in these tumors and contribute to poor breast cancer patient survival (Sarrio *et al.*, 2008). Likewise, relatively noninvasive ERα-positive MCF-7 cells express E-cadherin, consistent with a characteristic epithelial phenotype, and are classified as luminal subtype, whereas highly invasive ERα-negative MDA-MB-231 cells lack E-cadherin and are classified as basal subtype (Blick *et al.*, 2008). Furthermore, ERα directly correlates with E-cadherin in primary human breast tumors (Ye *et al.*, 2010). While EMT may enhance carcinoma cell invasion and subsequent dissemination which would increase metastatic potential, it is not synonymous with metastasis in all models. For example, Lou, *et al.* demonstrated that EMT alone was insufficient for spontaneous murine mammary carcinoma metastasis (Lou *et al.*, 2008). Yet, Weinberg and colleagues described the promotion of metastasis with loss of E-cadherin and a consequent EMT phenotype in transformed human breast epithelial cells (Onder *et al.*, 2008).

Our laboratory has previously demonstrated that exogenous IL-6 exposure induced an EMT phenotype in a panel of human ERα-positive breast cancer cells, which included E-cadherin repression and concomitant induction of Vimentin, N-cadherin, Snail, and Twist. In addition, ectopic expression of IL-6 in ERα-positive MCF-7 breast cancer cells promoted an EMT phenotype and enhanced invasiveness. Likewise, MCF-7 cells with ectopic Twist expression exhibit an EMT phenotype (Mironchik *et al.*, 2005), autocrine IL-6 production, and constitutive STAT3 activation (Sullivan *et al.*, 2009).

8. Therapeutic targeting of the IL-6/STAT3 pathway

IL-6 levels are increased in human breast tumors and breast cancer patient sera, and excessive IL-6, both circulating and within the breast tumor microenvironment, is associated with poor clinical outcomes in breast cancer. STAT3, a critical downstream mediator of IL-6 signaling, is constitutively activated in more than half of human cancers and promotes the expression of proliferative, anti-apoptotic, immune suppressive, and pro-angiogenic target genes, which all potentiate carcinogenesis. Whereas the IL-6 signaling network has been targeted in numerous autoimmune diseases and cancers, this therapeutic strategy has yet to be clinically employed for breast cancer. Increased preclinical reports have revealed novel

mechanisms underlying IL-6/STAT3 signaling in breast cancer cells such as enhanced growth, induction of EMT, multidrug resistance, and recruitment of peripheral fibroblasts. Taken together, accumulating preclinical and clinical data emphasize IL-6 as a highly attractive therapeutic target in breast cancer. It is therefore imperative that more work be done to evaluate current therapeutics and develop novel agents that target IL-6/STAT3 signaling in breast cancer models.

Multiple strategies could be utilized to target the IL-6/STAT3 pathway, but first and most obvious would be anti-IL-6 neutralizing antibodies. One such anti-IL-6 monoclonal antibody is Siltuximab (CNTO 328). The safety and efficacy of Situximab has been demonstrated in preclinical studies and phase I/II clinical trials of diverse human pathologies and malignancies including Castleman's disease (van Rhee *et al.*, 2010), multiple myeloma (Hunsucker *et al.*, 2011; Voorhees *et al.*, 2007), prostate cancer (Cavarretta *et al.*, 2007; Cavarretta *et al.*, 2008; Dorff *et al.*, 2010; Karkera *et al.*, 2011), renal cell carcinoma (Puchalski *et al.*, 2010; Rossi *et al.*, 2010), non-small cell lung cancer (Song *et al.*, 2010), and ovarian cancer (Guo *et al.*, 2010). Furthermore, IL-6R can be targeted with tocilizumab, an anti-IL-6R monoclonal antibody that has shown promising results in IL-6-driven autoimmune diseases (Tanaka et al., 2011) and was recently approved by the FDA for the treatment of rheumatoid arthritis. The promiscuous IL-6 coreceptor, gp130, also has an endogenous soluble form (sgp130) that exclusively inhibits IL-6 *trans*-signaling, thus preserving classical IL-6 signaling. Therapeutic sgp130 would potentially be more targeted toward breast cancer cells, which generally lack membrane-associated IL-6R and therefore utilize IL-6 trans-signaling through IL-6sR. Recombinant soluble gp130 (sgp130-Fc) has been shown to inhibit murine colon carcinogenesis (Becker *et al.*, 2004), suggesting that it may prove effective in breast cancer as well. Finally, a growing number of non-selective kinase inhibitors and recent focus on specific JAK and STAT3 inhibitor development will provide further insight into the roles of JAK and STAT3 in breast cancer.

9. Conclusions

Breast cancer is a heterogeneous disease and thus, highly variable across individual patients. This heterogenicity arises not only due to the diversity of genetic and molecular aberrations in primary breast cancer cells but also due to the diversity of cellular populations that inhabit the breast tumor microenvironment. Although IL-6 levels are higher in breast tumors and patient sera, the precise source of this IL-6 remains elusive. Importantly, many breast tumor stromal cells provide a paracrine source of IL-6 for breast cancer cells within the breast tumor microenvironment. In addition, certain clinical subtypes of breast cancers and research models, such as ERα-negative primary breast cancers and ERα-negative breast cancer cell lines, produce excessive IL-6 (Figure 2). Therefore, ERα-negative breast cancer cells may supply the tumor microenvironment with IL-6 by means of autocrine IL-6 production to exacerbate the poor prognosis associated with this clinical subtype. It will be critical to determine the specific cellular source of breast tumor-associated IL-6 to advance our understanding of this pleiotropic cytokine in breast cancer progression and metastasis. Moreover, this knowledge will facilitate the validation and subsequent clinical utility of current and novel targeted antagonists of the IL-6/STAT3 signaling network in breast cancer.
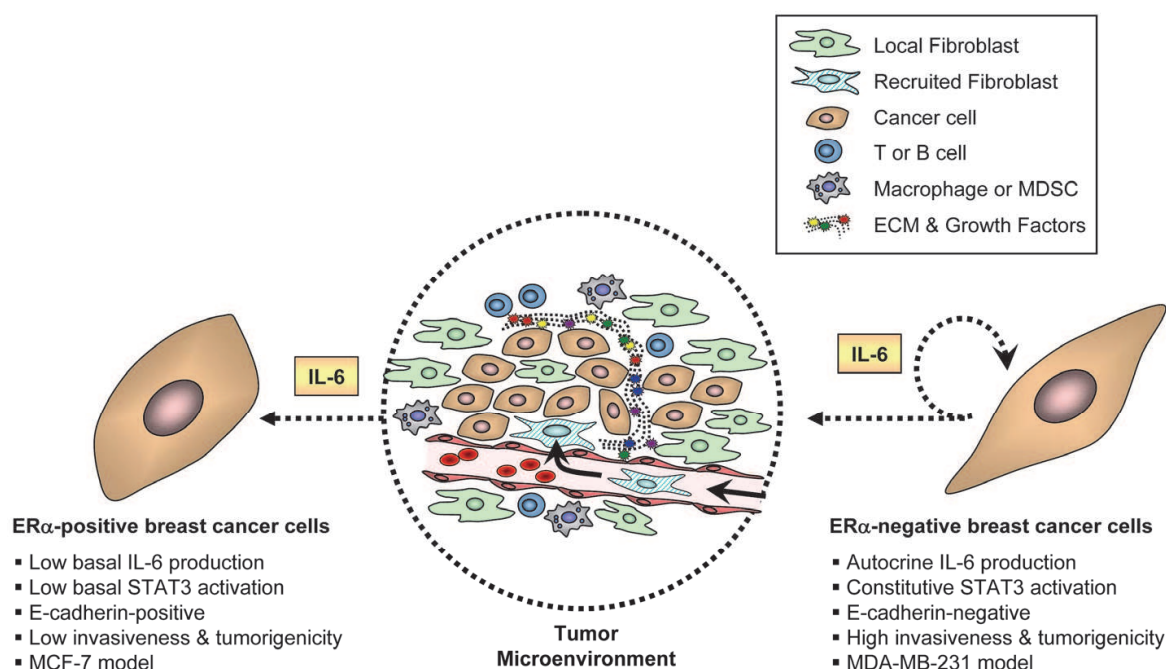
Fig. 2. Breast cancer cell ERα status dictates paracrine vs. autocrine IL-6 utilization.

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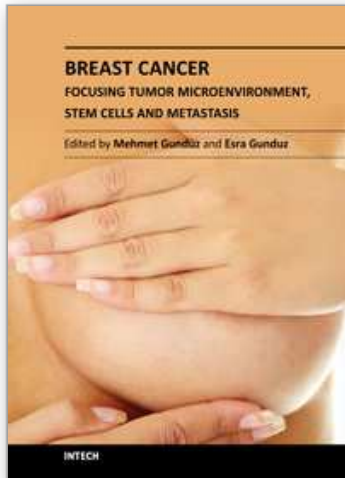
Breast Cancer - Focusing Tumor Microenvironment, Stem cells and Metastasis

Edited by Prof. Mehmet Gunduz

Cancer is the leading cause of death in most countries and its consequences result in huge economic, social and psychological burden. Breast cancer is the most frequently diagnosed cancer type and the leading cause of cancer death among females. In this book, we discussed characteristics of breast cancer cell, role of microenvironment, stem cells and metastasis for this deadly cancer. We hope that this book will contribute to the development of novel diagnostic as well as therapeutic approaches.

How to reference

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