IL-6 Cytokine Family in Breast Cancer

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The IL-6 cytokine family is a group of signaling molecules with wide expression and function across vertebrates. Each member of the family signals by binding to its specific receptor and at least one molecule of gp130, which is the common transmembrane receptor subunit for the whole group. Signal transduction upon stimulation of the receptor complex results in the activation of multiple downstream cascades, among which, in mammary cells, the JAK-STAT3 pathway plays a central role. The role of the IL-6 cytokine family—specifically IL-6 itself, LIF, OSM, and IL-11—as relevant players during breast cancer progression was summarized. The evidence indicating that this group of soluble factors may be used for early and more precise breast cancer diagnosis and to design targeted therapy to treat or even prevent metastasis development,particularly to the bone. Expression profiles and possible therapeutic use of their specific receptors in the different breast cancer subtypes are also described. In addition, participation of these cytokines in pathologies of the breast linked to lactation and involution of the gland, as post-partum breast cancer and mastitis, is discussed.

Keywords: breast cancer ; IL-6 ; tumor microenvironment ; mastitis ; LIF ; OSM ; IL-11

1. IL-6 in Breast Cancer

IL-6-induced signaling in breast tumors mainly triggers STAT3 activation. A positive correlation between phosphorylated STAT3 (pSTAT3) and IL-6 expression in primary breast tumors was first reported by Berishaj and collaborators, who also demonstrated that in vitro blockade of gp130 or IL-6 sequestration led to a decrease of pSTAT3 levels [1].

Involvement of IL-6 in breast cancer has been extensively reported over the years. Several groups from the 1980s to 2000s reported higher levels of this cytokine when sera from breast cancer patients and healthy women were compared [2]. Circulating IL-6 levels could be considered an independent prognostic marker given that higher IL-6 levels correlated with the stage of the disease [3]. Furthermore, in patients with untreated metastatic breast cancer, higher circulating IL-6 levels detected at the moment of diagnosis correlated with worse survival rates [4]. Conversely, a recent study shows that high levels of IL-6 and IL-10 detected in early stages of invasive breast cancer could be linked with good prognosis [5].

The role of IL-6 as a promoter of malignancy in breast cancer has been well established in different models and conditions. In 1989, a report was published demonstrating for the first time that IL-6 addition to cell culture medium enhanced motility as well as transition from cuboidal to fibroblastoid-like morphology of ER+ breast cancer cells. Importantly, these effects reverted upon cytokine removal [6]. A few years later another group shed some light on the involved mechanism demonstrating that incubation with IL-6 decreased E-cadherin expression in these cell lines [2]. Later studies confirmed that IL-6 increases migration and invasion capability of ER+ cells [7][8]. Therefore, it can be argued that even if ER+ breast cancer cells express low levels of IL-6, these cells can be highly susceptible to the presence of this cytokine in the microenvironment.

The association of IL-6 expression and breast cancer bad prognosis would not only be due to its relevance in tumor cell motility and epithelial-to-mesenchymal transition (EMT), but also to its essential role in cancer stem cell (CSC) self-renewal. It has been reported that IL-6 is upregulated in tumorspheres generated from patients' aggressive ductal breast carcinomas and from ER+ cell lines. Addition of this cytokine to those organoids promoted hypoxia-resistance, self-renewal, and invasiveness [8]. Stem phenotype induction upon ectopic IL-6 addition has been also demonstrated in TNBC cell lines and in primary cultures from human breast samples [9].

The role of the microenvironment on tumor initiation and progression has been increasingly studied in the last years. IL-6 is not only expressed and secreted by breast cancer cells, but also by diverse cell types that are part of the tumor microenvironment, such as myeloid-derived suppressor cells (MDSCs) [10], fibroblasts [11][12], lymphatic endothelial cells [13], and adipocytes [14]. Evidence of IL-6 release from a wide range of cell types and its association with breast cancer progression has been confirmed over the years and it has been described in different review articles [15][16]. Particularly, the contribution of adipose tissue has called the attention of various authors, since IL-6 serum levels positively correlated

with body mass in obese breast cancer patients [17]. This finding is especially relevant considering that survival rates of obese women with breast cancer are lower than those of non-obese breast cancer patients with similar tumor grades [18]. Besides, more advanced and higher-grade tumors exhibited increased IL-6 levels in the peritumoral adipose tissue [14]. In vitro studies support these clinically observed correlations, and it has now been established that adipocyte secreted IL-6 promotes migration of ER+ and ER− tumor cells [19].

Endocrine therapy (ET)—based on the use of tamoxifen, fulvestrant, and aromatase inhibitors—is the standard treatment for patients with ER+ breast tumors. However, in spite of its initial benefit, later resistance is very common and represents a hard clinical challenge [20]. Over the years, IL-6 has been increasingly linked to the acquisition of ET resistance in breast cancer patients [21]. Interestingly, ET resistant self-renewing CSCs from luminal tumors were characterized by the display of CD133hi/ERlo/IL6hi markers. These cells were IL-6/Notch dependent, and inhibition of these pathways induced re-expression of the ER and recovery of ET sensitivity [8].

IL-6 has been also implicated in resistance to trastuzumab treatment for women with HER-2/Neu breast cancer subtype. In these tumors it has been reported the induction of an IL-6 inflammatory feedback loop that leads to the expansion of CSCs, which in turn secrete high levels of this cytokine. Importantly, addition of tocilizumab, an anti-IL-6R antibody, is sufficient to revert this effect in vivo leading to tumor and metastasis inhibition [22]. Based on these data, a treatment that combines trastuzumab with tocilizumab is currently in a Phase I clinical trial for patients with metastatic trastuzumab-resistant HER2+ breast cancer (NCT03135171).

TNBC is defined by the lack of ER and PR, as well as low HER2 expression. Consequently, treatments for this BC subtype are limited to radiation therapy or chemotherapy [23]. In TNBC cell lines, inhibition of IL-6 and IL-8 expression dramatically reduced colony formation and cell survival in vitro and prevented tumor engraftment and growth in vivo [24]. Growth of TNBC xenografts has been inhibited by tocilizumab [13]. Interestingly, patients with TNBC showed increased presence of tumor associated macrophages and higher expression levels of IL-6 after surgery [25].

2. IL-6 Cytokine Family in Post-Partum Breast Cancer

Women diagnosed with breast cancer within 5 to 10 years of childbirth have significantly increased risk for metastatic recurrence [26][27]. It has been proposed that the higher probability of developing a metastatic disease during that period would be caused by mammary gland involution. During this process, which starts when milk production ends, either after birth in the absence of nursing or after weaning, dramatic tissue remodeling occurs in the mammary gland of all female mammals [28][29].

In female mice, expression of IL-6, LIF and OSM is low during lactation, but increases during involution together with the activation of Stat3 in mammary tissue. It has been shown that LIF is the cytokine responsible for activation of this transcription factor [30][31], while IL-6 induced ERK1/2 MAPK phosphorylation, which also plays a significant role during mammary regression [32]. Furthermore, OSM and OSMR are upregulated in response to STAT3 activation and the signaling triggered by this cytokine promoted the expression of metalloproteinases MMP3, MMP12, and MMP14, that are relevant for remodeling normal mammary tissue, but would also facilitate mammary tumor invasiveness [33].

Transgenic mice with mammary specific expression of the protease pappalysin-1 (PAPP-A), which is extensively overexpressed in breast cancers [34], have shown that extended lactation is protective against the oncogenic effect of PAPP-A, while abrupt halt of nursing increased its tumorigenic impact [35]. Interestingly, during mammary involution, PAPP-A expression is induced by IL-6 among other cytokines, and sudden interruption of lactation have triggered an increase in inflammation markers [36]. Therefore, possibly by ameliorating inflammation in the post-partum mammary microenvironment, extended lactation may be protective against breast cancer via suppression of PAPP-A [37].

3. IL-6 Cytokine Family in Mastitis

Mastitis, an inflammation of the mammary gland, is a worrying condition for nursing women, which may or may not be accompanied by infection. It is characterized by breast pain, swelling as well as redness, and can be followed by serious complications such as abscess and septicemia. Studies in different western countries revealed a relevant impact of this pathology with incidences of about 10–20% during the first 3 months post-delivery [38]. This pathology has also a great impact on cow milk production, since it causes premature weaning leading to reduced milk yield and quality. In addition, mastitis increases the cost of cattle management together with danger of antibiotic residues in commercialized milk [39]. This is due to the fact that in dairy cows, mastitis is usually caused by different bacteria including Gram-positive, e.g., *Staphylococcus aureus* (*S. aureus*) and Gram-negative, such as *Escherichia coli* (*E. coli*) [40]. The pathogenesis

caused by *E. coli* and other Gram-negative bacteria is often characterized by an acute inflammation, which, however, may eventually lead to pathogen clearance [41]. On the other hand, Staphylococci are the bacteria most commonly isolated from cases of subclinical mastitis [42]. Infection with these Gram-positive pathogens often causes mild signs of mastitis, but ineffective pathogen clearance frequently leads to chronic infection [43]. Importantly, infections by *S. aureus* are also a serious problem for women, because antibiotic resistance may cause eventual severity and difficulties to cure the illness [44].

Analysis of the transcriptome of primary bovine mammary epithelial cells after challenging them with heat-inactivated preparations of *E. coli* or *S. aureus* showed that the first rapidly and strongly induced expression of cytokines and chemokines while *S. aureus* elicited a retarded response. The genes that were most strongly upregulated by *E. coli* were clustered into a regulatory network with tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) in a central position. In contrast, the *S. aureus* response induced a functional network dominated by IL-6, although this cytokine would be also relevant for *E. coli* late response. Therefore, detection of IL-6 in milk has been proposed as a predictor marker for subclinical mastitis [45].

Interestingly, not only IL-6—but also its receptor, IL-6R—has been reported to be upregulated in the mammary glands of mastitic cows. This enhanced expression has been attributed to increased DNA methylation level during inflammation. Specifically, it has been proposed that the DNA methylation level in exon 2 of IL-6R could also be a potential biomarker for monitoring bovine mastitis [46]. Furthermore, Zhu et al., demonstrated an increase in the IL-6 mRNA in infected mammary lobes, but not in the mammary lobes that did not develop mastitis [47]. Similarly, comparing protein content in breast milk from human nipple single pores revealed that milk from mastitic lobes contained higher concentration of IL-6 than milk from healthy glands [48].

Plasma cell mastitis (PCM), also known as mammary ductal ectasia, is a special form of mastitis that typically occurs in young and middle-aged women at nonpregnant or non-nursing stages. IL-6/STAT3 signaling is activated in PCM and may play an important role in the pathogenesis of this illness [49]. Associated with this discovery, it has been proposed that sinomenine hydrochloride may achieve a therapeutic effect on PCM based on its anti-inflammatory and immunoregulatory properties, which are exerted by IL-6/JAK2/STAT3 pathway downregulation [50].

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