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overview

Protumor effects of proinflammatory mediators in breast cancer

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Abstract

Inflammation is defined as an enabling characteristic of malignant growth. Many proinflammatory mediators have protumor capabilities. In this review we focus on the protumor effect of cytokines and chemokines in breast cancer. We discuss the role of interleukin 1β, interleukin 6, tumor necrosis factor α, CCL2, CCL5, and CXCL12 and its receptor CXCR4 as typical mediators implicated in breast cancer progression. We also analyze the impact of transcription factor NF-κB. Proinflammatory mediators with protumor effects should be considered as therapeutic targets in breast cancer. It is challenging how to find optimal anti-cytokine and anti-chemokine regiments as a part of anticancer therapy.

INTRODUCTION

Malignant tumor is no longer considered to be a mass of neoplastic cells. There is intensive crosstalk between tumor and its surrounding tissue. Tumor, microenvironment is now considered to be important factor influencing tumor destiny *(1)*. Components of various processes are present in the tumor stroma: inflammation, specific immunologic response, angiogenesis and fibrinogenesis. The link between cancer and inflammation has been observed by Rudolf Virchow in 1863 *(2)*. More than century later, in the year 2011 Hanahan and Weinberg defined inflammation as an enabling characteristic of tumor growth *(3)*. Cancer and inflammation are connected by two pathways. Inflammation contributes to development of cancer and genetic alterations that lead to cancer stimulate the inflammatory processes resulting in tumor-favorable microenvironment. Inflammation influences all stages of carcinogenesis: initiation, promotion and progression *(1, 4)*. Mechanisms that relate inflammation and carcinogenesis are nowadays better defined and they include: production of ROS and RNS by activated inflammatory/immune cells which can cause DNA damage and contribute to genome instability and epigenetic alterations such as DNA methylation and histone modifications. Major mediators relating inflammation and cancer include cytokines, chemokines, COX-2, prostaglandins, NO and NF-κB *(1)*. In this review we will focus on two major mediators relating inflammation and cancer: cytokines and chemokines. We are going to discuss in more detail protumor effects of these mediators in breast cancer.

Protumor effects of cytokines

Interleukin -1ß (IL-1ß)

There are currently 11 members of IL-1 family of ligands and receptors *(5)*. In this review we will focus on IL-1β as it has been shown

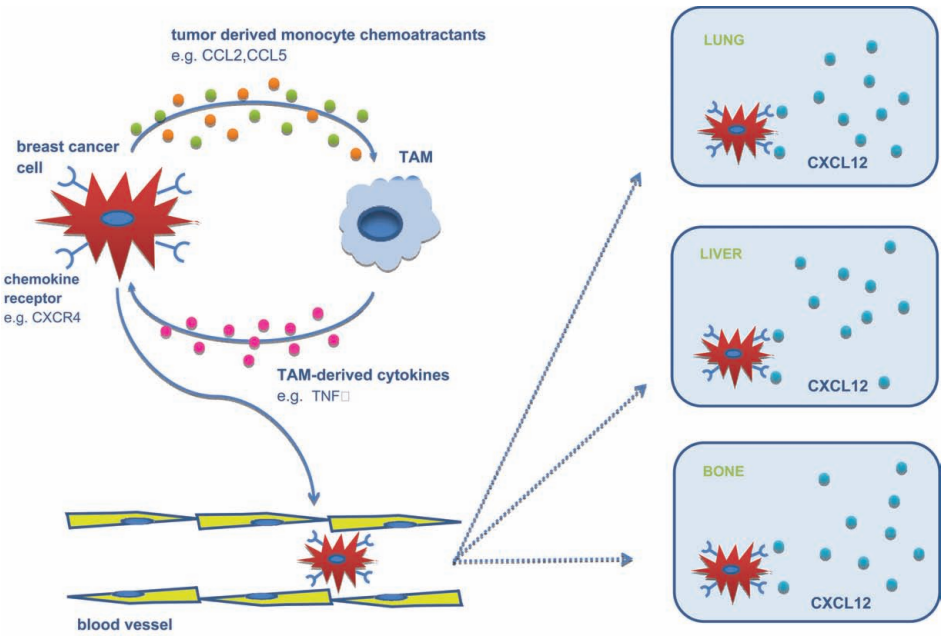

Figure 1. *Interaction between tumor cells, chemokines, cytokines and inflammatory cells. Two aspects of chemokine activities are shown: 1) Chemokines influence leukocyte infiltration. Tumor cells produce chemokines that attract monocytes (e.g. CCL2 and CCL5) resulting in an increase in tumor associated macrophages (TAM). TAM secrete cytokines like TNFα that further stimulate chemokine production. 2) Chemokines and their receptors play role in directing the homing of tumor cells to metastatic sites. Breast cancer cells express chemokine receptors (e.g. CXCR4) that enable them to interact with chemokines (e.g. CXCL12) expressed in distant organs.*

to be a primary mediator in context of chronic inflammation *(6)*. IL-1β induces cellular changes through binding of IL-1 receptor I (IL-1RI) at the cellular membrane and recruitment of coreceptor IL-2 RAcP. The second IL-1 receptor (IL-1RII) has no signaling capabilities but may act as a decoy receptor *(7)*. IL-1β has been shown to be expressed in many solid tumors, including breast cancer *(8)*. Patients with IL-1 producing tumors have worse prognosis than those with lower expression of IL-1 *(8)*. High serum levels of IL-1β correlate with recurrence in breast cancer patients *(9)*. IL-1β is produced both by breast tumor cells and by cells of the tumor microenvironment *(9)*. The concentration of IL-1β was reported to be significantly higher in invasive breast carcinomas than in ductal carcinoma *in situ* and benign lesions *(10)*. In the same study high IL-1β content was associated with tumor invasiveness and more aggressive tumor phenotype. Expression of IL-1β receptors was documented in human breast cancer cell lines *in vitro* and in a murine xenograft model *(11)*. Several studies related IL-1β to tumor migration and invasion. In the study of Wang et al. IL-1β has been linked to lower expression of E-cadherin and induction of matrix metalloproteinase-9 (MMP-9) *(12)*. Reduced expression of E-cadherin promotes cell migration; whereas expression of MMP-9 has a role in local extracellular matrix degradation and tumor invasion. It has been shown that IL-1 stimulates expression of endothelial adhesion molecules such as intracellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) *(6)*. ICAM-1 was shown to play a causal role in invasion of metastatic human breast carcinoma cell lines *(13)*. These findings confirm that some processes beneficial for host in the defense against exogenous stimuli (eg. facilitation of migration of neutrophils into tissue in case of bacterial infection) may be exploited by tumor and thus become harmful to the host. In various experimental models IL-1β was related to angiogenesis *(6, 14)*. IL-1β has ability to induce an angiogenic phenotype *(15)*. IL-1β acts via autocrine and/or paracrine mechanisms and may promote angiogenesis by regulating expression of angiogenic factors such as IL-8 *(6)*. IL-1β induces expression of hypoxia inducible factor-1α, a dominant transcription factor for vascular endotheliol growth factor (VEGF) in breast cancer cells *(16)*. IL-1β enhances inflammation by acting on other pro-inflammatory mediators and receptors. IL-1β was shown to activate a ROS-Src-MAPK-AP-1 pathway in breast cancer cells leading to increased COX-2 levels *(17)*. An autoamplification loop has been suggested: IL-1β secreted by macrophages enhances expression of IL-1β by both macrophages and breast cancer cells. IL-1β plays a role in crosstalk between cytokines and chemokines by inducing expression of chemokine receptors. In the study of Valdivia-Silva et al. IL-1β stimulation was followed by significant increase of CXCR4, CXCR2 and CX3CR1 expression in breast cancer cell line MCF-7 *(18)*. An interaction was shown between IL-1β and other factors that promote breast cancer: growth factors, growth factor receptors and steroid hormones. EGF ligand amphiregulin was found to regulate expression of IL-1β in breast cancer cells (SUM 149) *(19)*. In a mouse model IL-1β and fibroblast growth factor receptor 1 cooperate to induce COX-2 during early mammary tumorigenesis *(20)*. Speirs et al. reported transcriptional activation of ER alpha by IL-1β

in breast cancer cells *(21)*. Some authors analyzed some IL-1 family members, including IL-1ß in ER (–) and ER (+) breast cancer patient groups and found difference in cytokine profile *(22)*. IL-1*β* may have protumor effect by interfering with immune surveillance. IL-1*β* has been shown to be related to recruitment of suppressor cells called myeloid-derived suppressor cells (MDSC) *(23)*. These cells act as effective suppressors of $CD4^+$ and $CD8^+$ T lymphocytes. Mice with impaired IL-1ß signaling (IL-1R-deficient mice) have delayed accumulation of MDSC and slower growing mammary tumors with reduced metastatic potential *(24)*. Since IL-1 family members are secreted by adipose tissue these mediators are being explored as a potential link between obesity and breast cancer progression *(25)*.

Interleukin 6 (IL-6)

Interleukin 6 (IL-6) is another major proinflammatory cytokine that may contribute to the link between inflammation and cancer *(26)*. IL-6 exerts its effects in two ways. Classical IL-6 activation comprises IL-6 binding to IL-6 receptor on cell membrane (IL-6R). The IL-6/IL-6R complex then associates with signal-transducing membrane protein gp 130. In that way gp130 dimerisation is promoted and intracellular signaling that involves JAK is initiated. Alternative way by which IL-6 activates cells is called trans-signaling and comprises IL-6 binding to soluble form of the IL-6R (sIL-6R). Most genes that are targeted by IL-6 are involved in cell cycle progression and suppression of apoptosis *(27)*. By influencing anti-apoptotic pathways IL-6 might contribute to survival of DNA--damaged cells.

There are several mechanisms by which IL-6 might influence tumor invasion and spread. IL-6 stimulates epithelial cells to produce monocyte chemoattractant protein 1 (MCP-1) and colony stimulating factors *(28)*. IL-6 affects the process of invasion and metastasis by increasing the expression of matrix metalloproteinases (MMPs) *(29)*. IL-6 also up-regulates the expression of various adhesion molecules like ICAM-1 and endothelial leukocyte adhesion molecule-1 (ELAM-1) which are important for adhesion of tumor cells to endothelial cells, thus favoring tumor spread *(30)*.

In many studies serum circulating IL-6 has been shown to be a negative prognostic factor in breast cancer patients. IL-6 levels were higher in breast cancer patients than in healthy controls *(31)*. Higher IL-6 serum levels were observed in patients with recurrent disease than in non-recurrent cases *(32)*. Several authors found correlation between IL-6 serum levels and prognosis in metastatic breast cancer patients *(33)*. IL-6 expression in breast malignant tissue was found to be higher then in non--malignant tissue *(34, 35)*. However, Green et al. found no difference in expression between neoplastic breast tissue and normal breast tissue *(36)*. Studies *in vitro* showed that effects of IL-6 on breast cancer cells significantly vary depending on cell type *(37)*. However, it has been consistently reported that IL-6 promotes motility of breast cancer cells *(38, 39)*. IL-6 was shown to decrease cell adhesion by decreasing E-cadherin expression in breast cancer cells *(40)*. Sansone *et al*. reported up-regulation of IL-6 gene expression in mammospheres obtained from invasive breast carcinoma *(41)*. These authors also provided evidence that IL-6 regulates Notch-3 dependent signaling pathway thus promoting features that contribute to malignant phenotype (self renewal of cells, hypoxia survival and invasive potential) *(41)*. In some studies IL-6 was related to response to anticancer therapy. In the study of Conze et al. IL-6 autocrine production was shown to be important factor in determining multidrug resistance in breast cancer cells *(42)*.

Tumor necrosis factor α (TNFα)

TNFα was originally identified as an endotoxin-induced, macrophage-derived serum protein that has ability to induce necrosis of tumors *(43)*. TNFα belongs to a large family of proteins called "TNFα superfamily" *(44)*. TNFα transduces its signal through two distinct cell surface receptors: TNF-R1 and TNF-R2 *(45)*. TNFα receptor activation requires formation of multiprotein signaling complex leading to activation of transcriptional or an apoptotic pathway. In most situations the transcriptional pathway is activated *(37)*. TNFα is mainly synthesized by activated macrophages, NK cells, T cells, B cells and natural killer cells *(44)*. In contrast to high doses that are related to tumor destruction, exposure to low dose, chronic TNFα production is related to tumor promotion *(45)*. High TNFα levels were found in blood of cancer patients with various tumors, including breast cancer patients *(46)*. In several studies there was tendency for correlation between increased levels of TNFα expression and more advanced stage of breast cancer *(47, 48, 49)*. TNFα expression in inflammatory breast carcinoma was found to be related to higher tumor grade and lymph node involvement *(48)*. In breast tumors TNFα expression was found in breast tumor cells and in the cells of tumor stroma such as macrophages and endothelial cells *(48, 49)*. TNFα receptors were detected in breast tumors as well *(48, 49, 50)*. *In vitro* studies showed that TNFα promotes breast cancer cell proliferation and enhances estrogen-induced cell proliferation *(51, 52)*. The experiments *in vivo* confirmed that TNFα contributes to mammary tumorigenesis *(51, 53)*. NF-κB pathway was found to be critical for TNFα-induced tumor growth *in vivo* and *in vitro* (51). TNFα promotes tumor growth and progression by several mechanisms. TNFα induces various mediators that may influence tumor growth and progression. TNFα has been shown to be a potent inducer of monocyte chemoattractants such as CCL2 and CCL5 *(45)*. Positive loop may develop between tumor cells and tumor associated macrophages (TAM). Tumor cells produce monocyte chemoattractants such as CCL2 and CCL5. These chemokines induce monocyte migration to tumor sites resulting in accumulation of TAM. TAM express various cytokines including TNFα that further promote the expression of chemokines by tumor cells (Figure 1). Furthermore, TNFα has been shown to influence processes of motility and invasion. TNFα induces

expression of MMPs *(54)*. Besides being a potent stimulator of MMP-9 in monocyte cells, TNFα affects MMP production in breast cancer cells *(55, 56)*. Additionally, TNFα plays a role in epithelial-mesenchymal transition (EMT) of breast cancer cells- a process associated with promotion of invasion *(47, 57)*. Another protumor mechanism of TNFα is promotion of angiogenesis. TNFα simulates production of various angiogenic factors such as interleukin-8, VEGF and basic fibroblast growth factor *(58)*.This cytokine is involved in the process of bone resorption in metastatic breast cancer and presents an important factor that contributes to cancer cachexia in patients with malignant disease including breast cancer *(59, 60)*.

Protumor effects of chemokines

Chemokines are small proteins (8-10 kDa) with chemotactic activities important for leukocyte trafficking and homing. They are classified into four groups on the basis of the position of two cysteins that are adjacent to the amino terminus: CXC, CC, C and CX3C *(61)*. Chemokines bind to chemokine receptors which belong to the G-protein-coupled receptors. Most of those receptors bind more than one type of chemokine. However, some receptors bind only one ligand: e.g. CXCR4 is the only receptor for CXCL12 *(62)*. According to Ben-Baruch there are four major groups of chemokine activities in malignancy: 1) inducing the leukocyte infiltration to the tumor site; 2) directing the homing of tumor cells to metastatic sites; 3) regulating angiogenesis and 4) acting directly on tumor cells *(63)*.

CCL2 and CCL5

CC chemokine ligand- 2 (CCL2), also known as monocyte chemoattractant protein 1 (MCP-1) and CC chemokine ligand-5 (CCL5), also known as regulated on activation, normal T cell expressed and secreted (RANTES) have been intensively studied in breast cancer (reviewed in 64). Results of most studies suggest a protumor role of these chemokines in breast cancer. CCL2 and CCL5 have been detected in primary breast tumors, regional lymph nodes, metastases and serum of breast cancer patients *(64)*. CCL2 and CCL5 expression was significantly correlated with advanced tumor stage, early relapse and poor prognosis in breast cancer patients *(63)*. CCL5 was shown to be biomarker of disease progression in stage II breast cancer patients, especially when combined with absence of estrogen receptor α *(65)*. Both chemokines were found to be expressed in breast tumor cells of primary breast cancers *(66, 67, 68)*. Besides epithelial cells CCL2 and CCL5 were found in the cells of tumor microenvironment such as TAM and fibroblasts *(63)*. Studies of tumor cells in biopsies of breast cancer patients and human breast adenocarcinoma cell lines showed expression of receptors for CCL2 and CCL5 on tumor cells *(68)*. The fact that tumor cells express both chemokines and relevant receptors supports an autocrine mechanism of regulation. Both CCL2 and CCL5 have ability to influence the balance between leukocyte infiltrates in tumor microenvironment. These chemokines play a significant role in recruitment of monocytes leading to elevated presence of TAM in breast cancer *(64)*. Inhibition of CCL5 resulted in reduced TAM presence in mammary tumors *(69)*. Although initially considered as part of host defense TAM in breast tumors have been shown to have deleterious effects by releasing various tumor-promoting factors such as mediators that support growth, degradation of extracellular matrix and angiogenesis, and mediators that suppress immune functions. Some tumor-promoting activities of CCL2 and CCL5 do not fully overlap. For example CCL2 has more pronounced role in angiogenesis, while CCL5 is considered to be mainly an invasion-promoting factor. Besides having an indirect effect on angiogenesis by increasing TAM accumulation in breast tumor tissue, CCL2 has a direct effect by acting on endothelial cells to promote angiogenesis *(70)*. The effect of CCL5 as pro-invasive factor is related to its ability to induce breast tumor cell migration and to up-regulate the expression of MMPs *(71)*. Besides innate immunity (through effects on monocytes) CCL2 affects adoptive immunity as well. CCL2 has been shown to have negative effect on T cell effector function and to play a role in T helper cell polarization *(72, 73)*. The contribution of CCL2 and CCL5 to tumor growth was tested in various animal and *in vitro* models. Inhibition of CCL2 or CCL5 or their receptors resulted in reduction of tumor growth and reduced formation of metastases *(64)*. In the study of Soria et al. concomitant expression of CCL2 and CCL5 in breast tumors was found to be associated with more advanced stage of the disease *(68)*. In the same study relationship between CCL2 and CCL5 was shown: CCL2 up-regulated the release of CCL5 in experiments *in vitro (68)*. The same group of authors investigated expression of TNFα and IL-1β together with CCL2 and CCL5: the coordinated expression of the two cytokines and two chemokines was shown to be important for the disease course *(47)*. Expression of CCL2 and CCL5 as typical examples of protumor chemokines may influence the equilibrium between "pro" and "contra" tumor mediators in favor of those with tumor promoting functions.

CXCL12 and CXCR4

CXC chemokine ligand-12 (CXCL12), also known as stromal derived factor 1 (SDF-1) and its receptor CXC chemokine receptor 4 (CXCR4) play a crucial role in homing of hematopoietic stem cells, B-lymphocyte development and progenitor recruitment to sites of ischemic tissue damage *(74)*. CXCR4-CXC12 axis has been shown to be important for "homing" of cancer cells as well. Müller *et al.* analyzed human breast cancer cell lines and found the expression level of CXCR4 (and CCR7 as well) to be consistently increased in comparison to normal mammary epithelial cells. In the same study CXCL12 was expressed at high levels in the most common sites of breast cancer metastasis (lungs, liver, bone marrow and lymph nodes) and at low level in all other organs. Blocking the CXCR4 receptor decreased breast

cancer cell invasiveness *in vitro* and markedly reduced the number of lymph node and lung metastases in an *in vivo* mouse model *(75)*. These findings support "homing" theory of metastases- one of the theories that tries to explain why metastases to certain organs are not random (Figure 1). Several authors confirmed association of high level expression of CXCR4 with breast cancer metastases especially to lymph nodes *(76, 77, 78)*. In a large tissue microarray study CXCR4 expression was associated with breast tumor progression *(79)*. In the study of Holm et al. elevated expression of CXCR4 was related to recurrence in HER-2 negative breast cancer patients *(80)*. In some studies high level expression of CXCR4 was associated with poor overall survival in breast cancer patients *(81, 82)*. However, that was not confirmed by study of Kang *et al.* indicating that further analyses are needed to evaluate prognostic impact of CXCR4 *(77)*. CXCL12 is expressed at high levels in breast carcinoma associated fibroblasts *(83, 84)*. CXCL12 promotes the progression of breast cancer directly by enhancing tumor cell growth and indirectly by recruiting endothelial progenitor cells that are crucial for tumor angiogenesis *(83)*. In the study of Kang *et al.* CXCL12 expression significantly correlated with overall survival and incidence-free survival in breast cancer patients *(77)*.

NF-κB

NF-κB is an important transcription factor implicated in regulation of cytokines and chemokines. NF-κB regulates proinflammatory cytokines and these mediators act as stimulatory signals for NF-κB resulting in amplification that is often seen in inflammation. Such positive feedback mechanisms contribute to intensity of inflammation. In case of tumor growth these mechanisms may play a role in sustained tumorigenesis. NF-κB is a dimeric complex of Rel family proteins that functions as a transcription factor. It regulates more than 400 genes *(85)*. In resting state NF-κB is confined to the cytoplasm through its interaction with inhibitor of kappa B (IkB) proteins. Nucler translocation of NF-κB is crucial step in its activation. NF-κB may be activated by various exogenous and endogenous factors including proinflammatory cytokines, T-and B-cell mitogens, biological, physical and chemical stressors *(85)*. Constitutive activation of NF-κB has been reported for many cancers, including breast cancer *(86)*. Besides constitutive expression in tumor cells, according to Aggarwal and Gehlot, there are several lines of evidence that relate NF-κB to carcinogenesis: NF-κB regulates most of the genes linked to inflammation – some of those may have protumor effects; NF-κB regulates antiapoptotic genes, genes related to proliferation, invasion and angiogenesis; NF-κB has been linked to transformation; NF-κB is activated by numerous carcinogens while chemopreventive agents suppress its activation *(87)*.

In case of breast cancer NF-κB was related to progression of breast cancer to hormone-independent phenotype *(88)*. Subsequent studies have shown that NF-κB is activated in both hormone negative and hormone positive human breast cells *(89)*. In several studies NF-κB was related to more aggressive phenotype of breast tumors. Increased NF-κB activity was related to breast cancer overexpressing HER-2/neu, to poorly differentiated tumors and tumors with high mitotic counts *(90)*. NF-κB was reported to contribute to the unusual phenotype and aggressiveness of inflammatory breast cancer *(91)*. In study of ER-positive primary breast carcinoma, NF-κB was suggested to be a marker of high – risk subset of tumors *(92)*. Some drugs already used in breast cancer therapy like lapatinum inhibit the activation of NF-κB in HER-2-overexpressing breast cancer cells *(93)*. The activity of NF-κB has been implicated in promoting the chemoresistance of breast cancer *(94)*.

CONCLUSION

Experimental and clinical data support protumor effects of various cytokines and chemokines in breast cancer. These findings provide basis for considering these mediators as therapeutic targets. Some regiments directed against proinflammatory mediators are already in use in other medical indications *(6, 95)*. However, interfering with complex inflammatory network is not without potential harmful effects for physiological processes *(96)*. It is challenging how to find optimal anti-cytokine and anti-chemokine approach as a part of anticancer therapy. Some authors suggest targeting several mediators at the same time *(47)*. Another important problem is how to define the subgroup of patients that would have maximal benefit of such anti-inflammatory regiments. Anti-inflammatory agents are being explored in the prevention of cancer as well.

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