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Clinical and experimental approaches to the pathophysiology of interleukin-18 in cancer progression

Fernando Vidal-Vanaclocha · Lorea Mendoza · Naiara Telleria · Clarisa Salado · María Valcárcel · Natalia Gallot · Teresa Carrascal · Eider Egilegor · Jabier Beaskoetxea · Charles A. Dinarello

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Abstract
Summary Interleukin-18 (IL-18, interferon [IFN]-gamma-inducing factor) is a proinflammatory cytokine converted to a biologically active molecule by interleukin (IL)-1beta converting enzyme (caspase-1). A wide range of normal and cancer cell types can produce and respond to IL-18 through a specific receptor (IL-18R) belonging to the toll-like receptor family. The activity of IL-18 is regulated by IL-18-binding protein (IL-18bp), a secreted protein possessing the ability to neutralize IL-18 and whose blood level is affected by renal function and is induced by IFNgamma. IL-18 plays a central role in inflammation and immune response, contributing to the pathogenesis and pathophysiology of infectious and inflammatory diseases. Because immune-stimulating effects of IL-18 have antineoplastic properties, IL-18 has been proposed as a novel adjuvant therapy against cancer. However, IL-18 increases in the blood of the majority of cancer patients and has been associated with disease progression and, in some cancer types, with metastatic recurrence risk and poor clinical outcome and survival. Under experimental conditions, cancer cells can also escape immune recognition, increase their adherence to the microvascular wall and even induce production of angiogenic and tumor growth-stimulating factors via IL-18-dependent mechanism. This is particularly visible in melanoma cells. Thus, the role of IL-18 in cancer progression and metastasis remains controversial. This review examines the clinical correlations and biological effects of IL-18 during cancer development and highlights recent experimental insights into prometastatic and proangiogenic effects of IL-18 and the use of IL-18bp against cancer progression.

Keywords Interleukin-18 · Metastasis · Angiogenesis · Cancer progression · Immune response · Inflammation

F. Vidal-Vanaclocha (✉)
Department of Cell Biology and Histology,
Basque Country University School of Medicine and Dentistry,
Leioa, Bizkaia 48940, Spain
e-mail: gcpvivaf@lg.ehu.es

F. Vidal-Vanaclocha · L. Mendoza · N. Telleria · C. Salado · M. Valcárcel · N. Gallot · T. Carrascal · J. Beaskoetxea
Dominion Pharmakine, Bizkaia Technology Park,
Bldg 801, Bizkaia 48160, Spain

C. A. Dinarello
University of Colorado Health Sciences Center,
Denver, CO 80262, USA

E. Egilegor
Dominion Pharmakine, Bizkaia Technology Park,
Bldg 801, Bizkaia 48160, Spain

1 IL-18 in health and disease

1.1 Physiological aspects of interleukin-18

Interleukin-18 (IL-18, originally identified as interferon [IFN]-gamma-inducing factor) is a proinflammatory cytokine member of the interleukin (IL)-1 cytokine superfamily (that includes IL-1alpha, IL-1beta and IL-1 receptors) [1]. As IL-18 precursor protein contains an IL-1 signature-like sequence, interleukin-1beta converting enzyme (ICE-caspase-1) converts IL-18 to a mature biologically active 18.3-kDa form following cleavage of the propeptide. IL-18 binds to the cell through a specific receptor, IL-18R, belonging to the toll-like receptor family [2]. It is composed of IL-18Ralpha, which binds IL-18 with low affinity [3] and IL-18Rbeta, which does not bind IL-18 but together

with IL-18Ralpha forms the high-affinity IL-18 receptor and mediates signaling through pathways shared with the IL-1 receptor [4].

Under normal physiological conditions, IL-18 is produced by a wide range of cell types and exerts its biological activities at picomolar range. It is synthesized by activated monocytes, Kupffer cells and other organ-specific macrophages, osteoblasts, keratinocytes and other epithelial cells [5, 6]. IL-18R is also expressed on a variety of lymphoid cell types, including T lymphocytes, natural killer (NK) cells, peripheral CD19 positive B lymphocytes; and non-lymphoid cells, such as macrophages, endothelial cells, fibroblasts, melanocytes, cardiomyocytes, and numerous epithelial cells. In the central nervous system, resident cells also express IL-18 and caspase-1 constitutively, thus providing a local IL-18-dependent immune response. In the skeletal system, IL-18 promotes the catabolism of articular cartilage by up-regulating stromelysin and increasing proteoglycan release. It also suppresses osteoclastic bone-resorption mediated in part by IL-6 and induces the production of TNFalpha, granulocyte–macrophage colony-stimulating factor (GM-CSF), IFNgamma, CXC chemokines and nitric oxide by synovial fibroblasts. In the vascular system, IL-18 induces angiogenesis and promotes IL-8-mediated neutrophil chemotaxis via IL-1beta and TNFalpha production. It is also involved in leukocyte recruitment by up-regulating VCAM-1 through nuclear factor KappaB-dependent mechanisms. In the immune system, IL-18 stimulates Th1 differentiation, promotes secretion of IFNgamma, TNFalpha, and GM-CSF, and enhances the cytotoxicity of NK cells. IL-18 also elevates IgE due to IL-4 production from basophiles, mast cells and T cells. In contrast, administration of IL-18 in combination with IL-2 induces Th2 response in the host immune system, thus suggesting that IL-18 has multifunctional effects on the immune response, depending upon the environment [1, 5, 7–9].

1.2 Interleukin-18 binding protein

The activity of IL-18 is regulated by the IL-18 binding protein (IL-18bp), a constitutively expressed molecule that appears to be the natural inhibitor of IL-18 activity [7]. IL-18bp is a secreted 40-kDa glycoprotein possessing a high-affinity binding and ability to neutralize IL-18. IL-18bp was discovered in human urine and is excreted following glomerular filtration. With decreasing renal function, the concentration of IL-18bp in the circulation is elevated as compared with subjects with a normal renal function, and these elevated levels may result in a decreased IL-18 activity. IL-18bp expression is induced by IFNgamma, suggesting that feedback regulation also exists to control IL-18 activity [10]. It has been reported an exercise-induced decline in free IL-18 accompanied by up-regulation of IL-18bp. This may limit inflammatory response to exercise-induced tissue damage and may contribute to infections in athletes undergoing exhaustive exercise [11]. IL-18 and IL-18bp are, therefore, two opponents in the cytokine network and local concentrations of these two players determine the biological effects of IL-18 in a pathophysiological context.

1.3 Pathogenic aspects of IL-18

The availability of adequate methods for cytokine measurement has contributed to better understanding the immunopathophysiology of numerous diseases, and in some cases, to uncover that systemic level of certain cytokines, independently show promising correlations with disease stage and progression. IL-18 is a systemic molecular mediator that plays a role in homeostasis and that is modulated under many different disease conditions. It has been reported that serum IL-18 levels are higher in patients with advanced infectious diseases, acute graft-versus-host disease, chronic inflammatory bowel and liver diseases, and congestive heart failure and other cardiovascular diseases, among others, compared with normal controls [1, 7].

Under pathophysiological conditions, IL-18 plays a central role in inflammation and development of the adaptive immune response. IL-18 is an important mediator in the host response to infections. Viral infection induces IL-18 release from macrophages. Treatment of mice with exogenous IL-18 confers protection in mouse models of herpes simplex virus and VACV infections. Prophylactic treatment of *Candida albicans*-infected mice with recombinant IL-18 decreases mortality, improves outcome of disseminated candidiasis, and may prove useful as adjuvant immunotherapy of fungal infections [12]. However, IL-18 is a far more complex cytokine than previously thought. Inappropriate or unregulated IL-18 activity can result in or contribute to the damaging autoimmune response in diseases such as Crohn’s disease and rheumatoid arthritis [7, 13]. Other studies have highlighted a crucial role for IL-18 in mediating neuroinflammation and neurodegeneration in the central nervous system under pathological conditions such as bacterial and viral infections, autoimmune demyelinating disease, and hypoxic–ischemic, hyperoxic and traumatic brain injuries. IL-18 is also involved in the cytokine network associated with the inflammatory response after nerve crush during Wallerian degeneration of the rat nervous system [14].

IL-18 contributes to tissue damage during acute and chronic liver inflammation. IL-18 increases the susceptibility of liver endothelial cells to undergo apoptosis mediated by TNFalpha but not by Fas [15]. Interestingly, IFNalpha induces IL-18bp and, therefore, this anti-inflammatory property might account—together with its antiviral action—

for its clinical efficacy in chronic hepatitis C [16]. Cirrhotics have higher IL-18 levels than noncirrhotics and IL-18 increases with disease progression. IL-18bp plasma levels also paralleled the increase of IL-18 with disease progression, except in stage child C cirrhosis. IL-18 and IL-18bp levels were elevated independent of the etiology and correlated with laboratory parameters of inflammation, liver injury and severity of disease. IL-18bp may not be sufficient to counteract the overwhelming proinflammatory response in end stage liver disease [17].

The release of IL-18 into sera early in acute pancreatitis corresponds to disease severity. IL-18 induces nitric oxide (NO), which is involved in the pathophysiology of pancreatitis. Ueno et al. [18] found that IL-18 appears to protect the pancreas during early-induced acute pancreatitis in mice, probably through induction of NO release from an iNOS source.

Finally, human and rodent cancer cells can also produce IL-18, and circulating concentrations of IL-18 increase during metastatic disease as compared with levels in early cancer patients. On the other hand, it has been reported that constitutive or genetically manipulated production of IL-18 can enhance antitumor response, decrease tumor angiogenesis and improve survival. Thus, IL-18 has been proposed as a novel adjuvant therapy against cancer diseases. However, cancer cells can escape immune recognition, increase their adherence to the microvascular wall and induce production of angiogenic and growth factors via IL-18-dependent mechanism. Thus, the role of IL-18 in cancer progression and metastasis remains controversial. This review summarizes the effects of IL-18 on malignant processes, mainly emphasizing on the experimental systems that have been studied in our laboratory. In addition, the role of IL-18 is discussed in light of our results on the prometastatic and proangiogenic effects of IL-18.

2 IL-18 in patients with cancer

Given the immune-stimulating properties of IL-18, many studies have been conducted in patients with malignant tumors to determine serum IL-18 levels by enzyme-linked immunosorbent assay (ELISA), and to investigate the relationship between this cytokine and clinicopathologic factors and prognosis. In a pioneering study designed to compare IL-18 secretion in early and advanced cancer patients, Lissoni et al. [19] reported that a heterogeneous group of patients having metastases from lung and gastrointestinal tumors showed significantly higher IL-18 mean values with respect to both healthy controls and non-metastasized cancer patients. This preliminary study suggested for the first time that metastatic disease is characterized by enhanced IL-18 secretion.

With regard to tumors of the digestive system, Tsuboi et al. [20] reported that serum IL-18 levels were significantly higher in patients with esophageal carcinoma compared with healthy volunteers. IL-18 also increased in these patients as the pathologic stage progressed and correlated with tumor invasion and growth parameters. Kawabata et al. [21] reported that serum IL-18 level for patients with stage II and III gastric carcinoma was significantly higher compared with the mean level in healthy volunteers. Majima et al. [22] reported that IL-18 levels in the peritoneal cavity increased with gastric cancer progression and inversely correlated with survival. Human pancreatic carcinoma cells overexpressed IL-18 at mRNA and protein levels, and patients with low circulating levels of IL-18 also survived longer as reported by Bellone et al. [23]. Interestingly, serum IL-18 level decreased after patients underwent surgical resection of their gastric cancers and preoperative serum IL-18 level was identified as an independent postoperative prognostic factor in multivariate survival analysis [21]. Recently, we have determined IL-18 gene expression level by reverse transcriptase-polymerase chain reaction (RT-PCR) in hepatic metastases and unaffected peri-metastatic hepatic tissues from 40 patients with colon adenocarcinoma. IL-18 gene expression significantly increased in the hepatic tissue surrounding colon adenocarcinoma metastases as compared to control samples from non-metastasized human liver and to tumor samples obtained from metastatic tissue. As reported above, hepatic production of IL-18 occurs under many pathophysiological conditions and, consistent with the prognostic value of serum IL-18 level is the significant increase in the concentration of this cytokine detected in the hepatic blood during the experimental hepatic colonization of intrasplenically-injected B16 melanoma cells [24]. That tumor cells induce host cell production of IL-18 was further confirmed in primary cultured hepatic sinusoidal cells given the supernatant from cancer cells [25]. The low expression of IL-18 in hepatic colon carcinoma metastases is in agreement to Pages et al. [26] who observed decreased or abolished synthesis of IL-18 in colon adenocarcinoma samples from primary tumors as compared with normal colonic mucosa where synthesis of IL-18 by epithelial cells occurred. Similarly, Wen et al. [27] reported that IL-18 expression was decreased or absent in colon cancer, and that the lower the cancer was differentiated, the lower was the level of IL-18 expression in epithelium. However, if non-IL-18 producing cancer cells still can induce IL-18 production from host cells was not reported in these studies.

IL-18 is also up-regulated in patients with hepatitis C virus infection, which is the most common underlying disease in hepatocellular carcinoma. Asakawa et al. [28] investigated the role of IL-18 in hepatocellular carcinoma associated with hepatitis C virus infection. The IL-18

receptor was expressed in both the hepatocellular carcinoma tissues and two cell lines. NF-kappaB activation and the expression of Bcl-xL and xIAP mRNA were increased by recombinant IL-18. Moreover, IL-18 suppressed the apoptosis of hepatocellular carcinoma cells that was induced by etoposide *in vitro.* The overall survival rate was significantly worse in the IL-18R-positive patients than in the IL-18R-negative patients. In a Cox multivariate analysis, the expression of the IL-18R was found to be a significant predictor of a poor outcome in hepatocellular carcinoma patients. The expression of the IL-18R and an antiapoptotic mechanism involving NF-kappaB activation in hepatocellular carcinoma cells may be implicated in a poor patient outcome.

Similar to malignancies of the digestive system, Riedel et al. [29] reported that majority of patients with head and neck squamous cell carcinoma (HNSCC) had high concentrations of seric IL-18 as compared to the level in healthy controls, while levels of IL-10, IL-12, IFN-gamma did not significantly vary in these patients. Cortesina [30] reported that tumor specimens from patients with primary invasive HNSCC and derived cancer cell lines constitutively expressed IL-18 at the mRNA and protein levels indicating that HNSCC cells are a potential source of IL-18 cytokine. Park et al. [31] also reported high IL-18 expression in the squamous cell carcinoma cell line SK-MES-1. However, IL-18 protein was released as an unprocessed inactive 24-kDa form. Jablonska et al. [32] demonstrated markedly elevated serum levels of IL-18 according to the progression of the disease. Pratesi et al. [33] evaluated the correlation between functional single nucleotide polymorphisms in the promoter region of IL-10 and IL-18 genes and the virological and clinical characteristics in a large case series of Caucasian patients suffering from undifferentiated carcinoma of nasopharyngeal type, a tumor regularly associated with the Epstein–Barr virus. They found no evidence for involvement of IL-10 promoter polymorphisms alone in the genetic predisposition to this tumor. However, subjects with C/C or C/G combined IL-18 genotypes showed an increased risk of being with stages III–IV, suggesting that IL-18 genetic variants may represent a risk factor for tumor aggressiveness. Naumnik et al. [34] reported that serum IL-18 level in all patients with lung cancer was significantly higher compared with healthy volunteers. In non-small cell lung cancer group with stage IV the mean IL-18 level was significantly higher than those with stage IIIB.

IL-18 seems to be produced or secreted mainly by epithelial cells of lactating mammary gland but not of non-lactating mammary glands [35]. During breast carcinogenesis, Merendino et al. [36] reported that serum IL-18 levels were significantly higher in breast cancer patients with liver or bone metastases compared to patients without metastases and healthy donors. Similar results were reported by Gunel et al. [37] and Nouh et al. [38] in patients with metastatic breast carcinoma compared to non-metastatic patients and controls, suggesting IL-18 is an important non-invasive marker suspecting metastasis. They also observed [39] that serum IL-18 was significantly higher in patients with breast cancer when compared to the control subjects, and in patients whose tumor size was greater than or equal to 5 cm when compared to patients whose tumor size was less than or equal to 2 cm. Patients who were axillary lymph node negative had lower serum IL-18 levels when compared to patients with positive axillary lymph nodes. Moreover, serum IL-18 levels were significantly higher in patients with stage IIB or IIIA when compared to patients with stage I or IIA. Again, this suggests the usefulness of IL-18 as marker to predict prognosis of patients with breast cancer in complete remission after surgery, although long-term follow-up will be required to clarify this hypothesis.

With regard to ovarian cancer, Akahiro et al. [40] showed that serum IL-18 level in the ovarian cancer patients was significantly elevated compared with that in the normal controls and correlated with overall survival, but not with stage or histology. In a study on the relation between selected proinflammatory cytokine polymorphisms and ovarian carcinogenesis, Bushley et al. [41] reported that IL-18 G137C variant might be a marker for ovarian cancer progression or metastasis. Under normal physiological conditions, both IL-18 and ICE mRNA were expressed in normal cultured ovarian epithelial cells and IL-18 protein expression by the thin epithelial cell layer surrounding normal ovary [42]. In the same study, Wang et al. reported that normal ovarian epithelial cells released low but detectable amounts of mature IL-18 into the culture supernatant, which displayed IL-18-like biologic activity in functional assays. With regard to cancer cells, they detected IL-18 mRNA by RT-PCR in all human ovarian carcinoma cell lines tested and in 50% of tumor cell populations obtained from ovarian carcinoma patients. ICE mRNA was also expressed in a smaller fraction of samples (three of nine cell lines and three of eight samples from patients) and IL-18 protein was found in 7 of 13 ovarian carcinoma solid tumors by immunohistochemic analysis. Similar to HNSCC, they were able to detect abundant intracellular pro-IL-18 (24 kDa) in cancer cell lines by Western blotting, whereas the mature form of IL-18 was undetectable, irrespective of the presence of ICE mRNA and protein. Only pro-IL-18 was also found in the ovarian carcinoma cell supernatants, which did not display any IL-18 biologic activity in functional assays. These data suggest that mature biologically active IL-18 production is a feature of the normal ovarian surface epithelium lost during neoplastic transformation. Le Page et al. [43] used oligonucleotide-based DNA microarrays to identify potential markers

in primary cultures of ovarian cancer specimens compared with primary cultures of normal ovarian epithelia, which were further validated with RT-PCR, immunohistochemistry and ELISA. They reported that IL-18 was significantly elevated in tumor tissues and sera from patients with ovarian cancer. Moreover, in combination with FGF-2, and CA125, IL-18 significantly improved the specificity of detection as serum-based markers in epithelial ovarian cancer.

Many different studies on IL-18 have also been carried out in urologic tumors. With regard to bladder cancer, it has been reported that patients secreting high levels of IL-18 into the urine, during the first 12 h after intravesical bacillus Calmette–Guerin (BCG) instillation, have a higher chance of remaining disease-free than those secreting low levels, as shown by solid phase double ligand ELISA [44]. This suggests the potential value of low urine IL-18 levels to identify bladder cancer patients with high risk of disease recurrence and progression after BCG therapy. Consistent with the concept that IL-18 is the result of host defense against bladder cancer progression, Bukan et al. [45] reported that IL-18 levels were higher in patients with Ta stage than in patients with T1 and T2, T3, T4 stages, suggesting that IL-18 is associated to non-aggressive behavior of bladder cancer. More recently, it has been shown the importance of IL-18 followed by increases in Th1 cytokines, in the mechanisms of intravesical immunotherapy with BCG [46]. Moreover, a recombinant BCG strain that functionally secretes mouse IL-18 has been developed to augment BCG's immunostimulatory properties for bladder cancer immunotherapy [47].

Generally, IL-18 is significantly higher in bladder cancer patients when compared to the control subjects. However, because at least 50% of patients with a history of bladder cancer have recurrences, we have compared IL-18 gene expression in tumoral and non-tumoral mucosa samples obtained from non-recurrent and recurrent superficial bladder cancer patients (Ta and T1 of different G levels). In contrast to previously published data, our RT-PCR analyses showed that just 22% of the non-recurrent bladder cancers, as opposed to 60% of the recurrent bladder cancers had high IL-18 gene expression levels in tumor tissue samples. Interestingly, similar IL-18 gene expression levels were also detected in the normal bladder mucosa of same patient subgroups, which may account for the high levels of IL-18 secreted by these patients into the urine (Vidal-Vanaclocha et al., unpublished data).

Renal cell carcinoma patients also showed significantly higher serum IL-18 levels when compared to the control subjects [48]. In the same study, serum IL-18 levels were found to be similar in patients with T1 and T2, T3, T4 tumors, but patients with grade 3 and 4 tumors showed significantly higher serum IL-18 levels when compared to the patients with grade 1 and 2 tumors. With regard to prostate cancer, Lebel-Binay et al. [49] reported that clinically localized prostate cancers frequently presented with cancer cells producing IL-18, as determined by immunohistochemistry. Consistent with these findings, they showed that prostate tumor cell lines PC-3, DU 145 and LNCaP synthesized the immature form of IL-18 and that IFN-gamma produced in prostate cancers induced ICE mRNA and IL-18 secretion from cancer cell lines, which was inhibited by ICE inhibitor. IFN-gamma also induced IL-18 secretion of the poorly differentiated cell line PC-3. Interestingly, PC-3 and DU 145, but not the well-differentiated cell line LNCaP, expressed IL-18R alpha protein and transcripts for IL-18R beta suggesting that IFNgamma-induced IL-18 could act as an autocrine/paracrine factor for the tumor.

Zhang et al. [50] investigated the levels of IL-18 in the bone marrow of patients with hematological diseases to determine their pathogenic significance. They detected that IL-18 mRNA levels were higher in the patients with leukemia and other malignant hematological diseases than in normal donors. Immunohistochemical method confirmed the presence of IL-18 protein in most acute myeloid leukemia cases with positive transcription. In addition, IL-18 antisense oligodeoxynucleotides clearly inhibited the growth of J6-1 and HL-60 cells in a dose-dependent manner suggesting that IL-18 might be involved in the proliferation of certain leukemic cells *in vivo* through an autocrine mechanism. They also used RT-PCR to assess levels of IL-18 mRNA in bone marrow mononuclear cells to explore the clinical significance of IL-18 in patients with acute myeloid leukemia. Either disease status, age or CD34 expression were found to significantly correlate with the expression of IL-18 and a significant difference in IL-18 gene expression was obtained between the high risk group and the intermediate risk group suggesting that IL-18 gene over-expression might reflect the convergence of several important unfavorable prognostic factors in acute myeloid leukemia. To establish a possible functional relationship between IL-18 and MMPs in myeloid leukemia, Zhang et al. [51] used semi-quantitative PCR and zymographic analysis to examine whether IL-18 stimulates human myeloid leukemia cell line HL-60 to produce MMPs and/or specific tissue inhibitors (TIMPs), and to degrade extracellular matrix gel *in vitro*. In the invasion assay IL-18 significantly up-regulated transmigration of HL-60 cells, which in turn was inhibited by a synthetic MMP inhibitor *O*-phenanthroline, anti-MMP-9, anti-MMP-2 as well as anti-IL-18 monoclonal antibody, respectively, suggesting that induction of gelatinases by IL-18 leads to extracellular matrix degradation. Moreover, IL-18 could significantly increase MMP-9 but not MMP-2 production, slightly up-regulate TIMP-1, and clearly induce TIMP-2 secretion, suggesting

that IL-18 may in part play a role in the clinical aggressiveness of human myeloid leukemia by stimulating MMP-9 production. Alexandrakis et al. [52] determined serum levels of IL-18 in 65 newly diagnosed myeloma patients. IL-18 was significantly higher at stage III in comparison to stages II and I. Cytokine level significantly decreased after treatment. In survival analysis, higher levels of IL-18 were associated with a poorer prognosis. They concluded that increased serum IL-18 in myeloma patients correlates with advanced disease, increased levels of angiogenic cytokines and worse survival. Similar to matrix metalloproteinases (MMP-9/-2), IL-18 was overexpressed in some hematologic malignancies such as acute myeloid leukemia, which is associated with a poor clinical outcome. Airoldi et al. [53] evaluated IL-18 expression and production in tonsil naive, germinal center, and memory B cells and in their presumed neoplastic counterparts by RT-PCR and ELISA. They found that: IL-18 mRNA was expressed in tonsil naive, germinal center, and memory B cells and secreted as bioactive IL-18 by naive and germinal center, but not by memory B cells; IL-18Ralpha was detected on the surface of naive, germinal center, and memory B lymphocytes, and IL-18Rbeta was detected on germinal center and memory, but not naive, B cells; mantle zone, follicular, marginal zone, Burkitt lymphoma (BL), and B-cell chronic lymphocytic leukemia cells expressed IL-18 mRNA, but B-cell chronic lymphocytic leukemia and Burkitt lymphoma cells did not produce bioactive IL-18; and lymphoma B cells displayed heterogeneous expression of either or both IL-18R chain mRNA. In contrast, B-cell chronic lymphocytic leukemia cells expressed both IL-18R chains at the mRNA and protein levels. Thus, deregulated expression of IL-18 and/or IL-18R in chronic B-cell lymphoproliferative disorders may sometimes contribute to tumor escape from the host immune system. Recently, Mazodier et al. [54] observed that the concentration of IL-18 was highly increased in hemophagocytic syndrome but not in control patients. In contrast, concentrations of its natural inhibitor, the IL-18bp, were only moderately elevated, resulting in a high level of biologically active free IL-18 (4.6-fold increase compared with controls). Free IL-18 but not IL-12 concentrations significantly correlated with clinical status, biologic markers of hemophagocytic syndrome and also with markers of Th-1 lymphocyte or macrophage activation. Despite high IL-18 elevation, *in vitro* NK-cell cytotoxicity was severely impaired in these patients. They concluded that a severe IL-18/IL-18bp imbalance results in Th-1 lymphocyte and macrophage activation, which escapes control by NK-cell cytotoxicity and may allow for secondary hemophagocytic syndrome in patients with underlying diseases such as cancer.

In summary, irrespective of its biological activity, IL-18 concentration significantly increases in the blood of the majority of cancer patients (Table 1). Its production is a pathophysiologic feature of cancer connecting inflammatory and immune responses to cancer progression. However, the regulatory pathways for IL-18 production by both cancer cells and tumor-induced host cells, and its mechanisms of action remain to be determined in cancer patients. In addition, the majority of studies on the IL-18 level in cancer patients did not provide information about the concentration of its antagonist (IL-18bp) and IL-18-inducible cytokines (IFNgamma, TNFalpha, CSF-GM, etc) in same blood samples. Because IL-18 and IL-18bp levels correlate with parameters of inflammation, tissue injury and the severity of disease in other clinical models [17, 54], it would be essential to know whether or not IL-18bp level is sufficient to counteract the pleiotropic effects of IL-18 in the different stages and clinical conditions of cancer patients. On the other hand, according to these studies, IL-18 might be a candidate seric marker to prognosticate and monitor the clinical course of many cancer types. More importantly, despite the expected favorable event in the course of the disease, IL-18 could represent a marker for metastatic progression in some cancer types. At the same time, this concept raises the possibility that IL-18 may offer an advantage for tumor development in patients with certain types of cancer. For this reason, if either administration of IL-18 or modulation of IL-18 production has beneficial effects deserves further clinical investigation. In the next sections of this review, we summarize the experimental data supporting pro- and anti-tumoral effects of IL-18, emphasizing experimental systems that have been studied in our laboratory.

3 Inhibition of cancer progression by IL-18 effects

3.1 Immune response-dependent antineoplastic effects of IL-18

IL-18 in the presence of IL-12 stimulates the lytic activity of NK cells, induces IFN-gamma and GM-CSF by activated T cells, stimulates T cell proliferation, and induces Th1 cell responses [1] which are essential immune responses in the host defense against cancer. Several studies have, therefore, determined the possible correlations between IL-18 effects and anti-tumor immunity in experimental models of cancer. In some cases, altered expression and function of IL-18 and IL-18R were involved cancer progression. For example, Kobashi et al. [55] evaluated cellular immune responsiveness—as IL-18-induced IFN-gamma production from peripheral blood mononuclear cells (PBMCs)—in patients with advanced cancer and in normal controls. Supernatant levels of IFNgamma were detected at 2 h after PBMCs culture and markedly increased thereafter in healthy volunteers. In contrast, IFNgamma

Table 1 IL-18 and IL-18R status in cancer patients and their clinical correlations

Tumor type	IL-18 and IL-18R status	Clinical correlations and prognostic value	References
Esophageal carcinoma	High seric concentrations	Clinical stage Tumor invasion and growth	[20-22]
Gastric carcinoma	High seric concentrations	Increased levels in peritoneal cavity Risk of cancer progression Poor survival Postoperative prognostic factor	
Pancreatic carcinoma	High seric concentrations	Poor survival	[23]
Colon adenocarcinomas	Increased gene expression in perimetastatic hepatic tissue Low expression in primary tumors	Risk of metastasis recurrence	[26, 27]
Hepatocellular carcinoma	High expression of IL-18R	Poor survival Poor clinical outcome	[28]
Head and neck squamous cell carcinoma	High seric concentrations High level in tumor tissues	Risk of cancer progression	[29-32]
Lung cancer	High seric concentrations	Clinical stage	[34]
Breast cancer	High seric concentrations	Clinical stage Risk of metastasis recurrence	[36-39]
Ovarian cancer	High seric concentrations Elevated level in tumor	Poor survival	[40, 43]
Bladder cancer	High urine concentrations High level in tumor	Disease-free time Non-aggressive behavior	[44, 45]
Renal cell carcinoma	High seric concentrations	Clinical stage	[48]
Prostate cancer	High level in tumor tissues	Localized disease	[49]
Leukemia	High IL-18 mRNA levels	Clinical aggressiveness Poor clinical outcome	[50]
Myeloma	High seric concentrations	Clinical stage Poor clinical outcome and survival	[52]

production in cancer patients was not detected during the same culture period. They also measured IL-18-stimulated IL-12 production in healthy volunteers and null response was observed in cancer-bearing patients. Both mRNA levels of IL-18R and IFNgamma were significantly decreased in cancer-bearing patients compared with normal controls suggesting that IL-18 responsiveness for IFN-gamma production in cancer-bearing patients was impaired. Impaired IL-18 responsiveness in PBMCs from cancer-bearing patients was, at least in part, ascribed to a drastic decrease of NK cells and T lymphocytes which constitutively and highly express IL-18R, and also attributed to null production of IL-12 which up-regulates IL-18R. In other cases, despite the apparent expression of IL-18 by cancer cells, its production as mature biologically active IL-18 is a feature of the normal cells frequently lost during neoplastic transformation as for example in ovarian, bladder and colon carcinoma cells [26, 42]. Therefore, whether or not the capability of normal and cancer cells to produce IL-18 represents an antitumoral factor preventing cancer progression should be investigated.

On the other hand, certain DNA viruses encode a variety of immune defense proteins, some of which bind to cytokines and neutralize their activities. Ectromelia, vaccinia, and cowpox viruses [56] and poxviruses [57] such as *Molluscum contagiosum* virus (MCV) encode secreted IL-18 binding proteins. The MCV IL-18bp binds human and murine IL-18 with high affinity and inhibits IL-18-mediated IFNgamma production. Lee et al. [58, 59] demonstrated that both E6 and E7 oncoproteins of human papillomavirus (HPV) 16 inhibit IL-18-induced IFN-gamma production in human peripheral blood mononuclear and NK cells. Their results suggest that down-modulation of IL-18-induced immune response caused by these oncoproteins is one of the mechanisms underlying immune escape in HPV-infected cervical lesions including cervical cancer cells.

That immune-stimulating effects of IL-18 have antineoplastic properties has long been reported in experimental models. For example, Jonak et al. [60] administered IL-18 at high doses as a single agent for prolonged periods in mice bearing MOPC-315 tumors. They reported an effective Th1 antitumor immune response and tumor regression in all animals. Yamashita et al. [61] investigated the effects of mouse recombinant IL-18 on the lodging and subsequent growth of multiple myeloma cells in the bone marrow. An intravenous injection of human multiple myeloma cell line (ARH-77 cells) into mice with severe combined immunodeficiency disease which results in lodging of tumor cells in the

bone marrow of thoracic and lumbar vertebrae was used. In this model, the subsequent growth of cancer cells destroys bone, invading the spinal cord and surrounding tissues, and the mice show hind leg paralysis. Using this model, the rate of hind leg paralysis and the tumor area significantly decreased when IL-18 was daily injected from day 6 after tumor cell injection. This antitumor effect of IL-18 was ascribed to its action on the activation of NK cells because IL-18 exerted no significant effect when anti-asialo GM1 antiserum was simultaneously injected to deplete the NK cell activity. Okamoto et al. [62] examined the usefulness of IL-18 in the treatment of osteosarcomas. Daily intraperitoneal injection of mouse recombinant IL-18 suppressed the growth of Dunn osteosarcoma cells transplanted subcutaneously into syngeneic C3H mice. This IL-18-induced suppression was not affected by simultaneous treatment with anti-asialo GM1 serum. However, IL-18 failed to suppress the growth of Dunn osteosarcoma cells transplanted into BALB/c-nude mice devoid of T lymphocytes or C3H-gld/gld mice deficient in functional FasL. Interestingly, treatment of C3H mice with IL-18 enhanced the cytotoxic activity of CD8+ T lymphocytes against Dunn osteosarcoma cells and antimouse Fas antibody showed cytotoxicity against these cancer cells in a dose-dependent manner *in vitro*. These results indicate that IL-18 inhibits the growth of Dunn osteosarcoma cells *in vivo* by enhancing the cytotoxic activity of CD8+ T lymphocytes through the Fas-L–Fas system.

IL-18 has also been used to decrease the dosage of IL-2 while maintaining its antitumor therapeutic effects are being made. For example, Aria et al. [63] reported that IL-18 combined with IL-2 potentiates *in vivo* NK cell activity and contributes to inhibition of tumor metastasis without inducing significant toxicity. Son et al. [64] observed that combined use of IL-18 and low-dose IL-2 synergistically promoted *in vitro* proliferation of NK cells with up-regulation of IL-2 receptor-alpha and synergistically stimulated cytolytic activity and IFNgamma production by these cells. Furthermore, intratumoral injections of these two cytokines completely eradicated established subcutaneous tumor and induced CD4+-dependent memory in a MCA205 murine tumor model, suggesting that combined administration of IL-18 and low-dose IL-2 could be a model for cancer immunotherapy. Redlinger et al. [65] reported that coadministration of low-dose IL-2 plus IL-18 induced a potent primary response to murine neuroblastoma likely caused by activation of NK cells in the tumor microenvironment. However, this combined cytokine therapy was unable to induce sustained immunity to rechallenge.

IL-18 in combination with IL-12 also resulted in a more potent antitumor response compared with each cytokine alone [66, 67]. The IL-12/IL-18 skewing of effector T cells towards a type 1 response also resulted in more potent antitumor reactivity *in vivo* and was found to be NFkappaB-dependent in cultured tumor-draining lymph node cells [68]. However, Osaki et al. [69] also found that administration of IL-12 and IL-18 was associated with lethal organ damage, attributed in part to extremely high levels of host-induced IFN production. In a different approach, the IL-18 gene was transferred into dendritic cells, either alone [70, 71] or simultaneously with the IL-12 gene [72] resulting in more effective antitumor responses associated with dendritic cell-based vaccines.

Finally, several agents appear to modulate the multiple mechanisms of immune response-dependent anti-tumoral effects of IL-18. Hegardt et al. [73] investigated the role of NO produced by adherent spleen cells in the systemic immunosuppression developing in tumor-bearing hosts. They demonstrated that the competitive NO synthase inhibitor L-NAME could partially counteract the suppression *in vitro* and suggested that NO synthase inhibitors in combination with IL-18 could be useful tools to enhance anti-tumor immune responses and the efficiency of immunotherapies. Iigo et al. [74] demonstrated a significant increase in caspase-1 activity, IL-18 and IFNgamma in the small intestine after oral administration of bovine lactoferrin, and suggested that anti-metastatic effects of lactoferrin treatment are possibly due to potentiation of the killing activity of T and NK cells against tumor cells. Merendino et al. [75] observed that serum IL-18 levels increased significantly with respect to baseline in patients receiving adjuvant chemotherapy with 5-fluorouracil (5-FU) and folinic acid. Carbone et al. [76] also reported that adjuvant polychemotherapy including 5-FU significantly increased serum levels of mature, bioactive IL-18 in pancreatic carcinoma patients. Interestingly, *in vitro* treatment of human pancreatic cancer cells with 5-FU induced caspase-dependent processing of pro-IL-18 leading to the secretion of biologically active IL-18, as shown by induced IFN-gamma production by activated T cells.

3.2 Immune response-independent anti-neoplastic effects of IL-18

Cancer cells interact with its microenvironment and each profoundly influences the behavior of the other. Anti-tumor immune response is only part of the story in the control of cancer cell behavior and development. There are other multiple tumor-host cell interactions that either inhibit or permit, and even encourage, cancer progression. IL-18 has been found to have several biological effects involving non-lymphoid cells. IL-18 can act on endothelial cells and pericytes, and several studies have suggested that antineoplastic effects of IL-18 may in part be mediated by anti-angiogenic mechanisms. For example, Coughlin et al. [77] reported that SCK cells expressing IL-12 or IL-18 were less tumorigenic and formed tumors more slowly than control

cells via systemic inhibition of angiogenesis. Cao et al. [78] reported that IL-18 inhibited FGF-2-stimulated proliferation of endothelial cells *in vitro* and suppressed the FGF-induced corneal neovascularization by systemic administration in mice. IL-18 produced a significant suppression of the growth of murine T241 fibrosarcoma *in vivo* and immunohistochemical studies of tumor tissues revealed hypovascularization of IL-18-treated tumors, while T241 fibrosarcoma cells were insensitive to IL-18 at concentrations that significantly inhibit endothelial cell proliferation. Consistent with these findings, Shimamura et al. [79] suggested that antitumor activity of bovine lactoferrin might be partly mediated by IL-18-dependent angiogenesis inhibition. On the other hand, Nakata et al. [80] reported that systemic daily administration of recombinant IL-18 inhibits the development of experimental osteolytic bone metastasis produced by an intracardiac injection of MDA-231 human breast cancer cells. This systemic treatment schedule with IL-18 also significantly inhibited the number and total area of osteolytic bone metastasis by RWGT2 human lung cancer cells in nude mice. The anti-osteoclastogenic effect of IL-18 occurred through the suppression of osteoclastic bone-resorption mediated in part by IL-6 [81].

Nakamura et al. [82] investigated the effect of IL-18 on metastasis of highly metastatic LM8 mouse osteosarcoma cells in nude mice treated with anti-asialo GM1 serum to exclude anti-tumor actions of IL-18 through activation of T and NK cells. Injection of LM8 cells that do not express IL-18Rbeta resulted in the formation of pulmonary and hepatic metastatic foci. Five daily injections of IL-18 before and after the cancer cell injection resulted in marked metastasis decrease. The retention of LM8 cells in the lung 24 h after their injection was also reduced by the pretreatment of mice with IL-18. Pretreatment of mice with IL-18 for 5 days before LM8 cell injection markedly decreased metastases. Serum obtained from mice pretreated with IL-18 for 5 days suppressed mobility of LM8 cells but IL-18 itself did not, which suggests that IL-18 inhibits metastasis of LM8 cells partly by inducing a factor(s) in the host that suppresses cell mobility.

In summary, constitutive or genetically manipulated production of IL-18 can enhance antitumor response and improve survival under experimental conditions. This depends in part on immune-stimulating effects of IL-18, alone or in combination with other cytokines (IL-2, IL-12) and miscellaneous agents (NO, 5-FU). On the other hand, immune response-independent antitumor effects such as osteoclastogenesis inhibition and hypovascularization of IL-18-treated tumors may concurrently contribute to antitumoral effects of IL-18. Based on these data, IL-18 has been proposed as a novel adjuvant therapy against cancer diseases. GlaxoSmithKline, under license from Hayashibara, is developing SB-485232, a recombinant human interleukin-18 cancer immunotherapy for the potential treatment of immunologically sensitive cancers. The compound is currently undergoing phase II clinical trials [83].

4 Activation of cancer progression by IL-18 effects

Although many experimental investigations have described IL-18 as an antitumor cytokine that enhances NK cell activity and even induces immune-independent anti-tumor activities with no apparent adverse effects, clinical studies have also reported negative aspects of IL-18 in patients with cancer. These are consistent with experimental evidences on the tumor and host production of IL-18 upregulating tumorigenic, angiogenic and even prometastatic mechanisms of cancer cells. Thus, whether IL-18 is a useful tool against cancer progression needs further attention.

4.1 Role of host cell-derived IL-18 in the prometastatic microenvironment of tumor-induced inflammation

The mechanism of microvascular arrest of circulating cancer cells, which is a critical step in metastasis, appears to be facilitated by tumor-derived proinflammatory factors that increase endothelial cell adhesion receptors for cancer cells [84]. Using intrasplenically injected B16F10 melanoma cells, we showed that the expression of VCAM-1 significantly increased in hepatic sinusoidal endothelium (HSE) cells over physiologic baseline within the first 24 h of metastatic cancer cell infiltration in the liver [85]. This correlated with increased *in vitro* adhesion of B16 cells to HSE cells isolated from B16F10 cell-injected mice. *In vivo* VCAM-1 blockade with specific antibodies before B16 cell injection decreased microvascular retention of luciferase-transfected B16F10 cells by 85%, and metastasis development by 75%, indicating that VCAM-1 expression on tumor-activated HSE cells had a prometastatic contribution in the liver. Because VCAM-1 expression is oxidative stress-inducible, recombinant catalase was *in vivo* administered, resulting in a complete abrogation of both VCAM-1 expression and B16 cell adhesion increases in HSE cells isolated from B16 cell-injected mice. Catalase also abrogated the proadhesive response of HSE cells to B16F10-conditioned medium (B16M-CM) *in vitro*, although this did not affect the concomitant release of major proinflammatory cytokines by HSE cells [85].

HSE cells treated with B16F10-CM released IL-18 via TNF-alpha-dependent IL-1beta *in vitro*. In turn, hydrogen peroxide production from B16F10-CM-treated HSE cells was regulated by IL-18. Finally, liver-infiltrating B16F10 cells activated their adhesion to HSE through a sequential process involving TNF-alpha-dependent IL-1beta, which induced IL-18 to up-regulate VCAM-1 via hydrogen peroxide [85]. The pivotal position of IL-18-induced

hydrogen peroxide was further supported by the fact that incubation of HSE cells with nontoxic concentrations of hydrogen peroxide directly enhanced VCAM-1-dependent B16F10 cell adhesion *in vitro* without proinflammatory cytokine mediation, which emphasizes the key role of oxidative stress in the pathogenesis of liver inflammation and IL-18-dependent metastasis.

We also studied the role of the IL-1beta-converting enzyme (ICE) in B16 melanoma metastasis in mice. To differentiate between the role of IL-1beta and IL-18 in ICE-deficient mice, we compared B16 metastasis after intrasplenic injection of B16 cells into mice deficient in ICE or IL-1beta. In addition, the role of IL-18 in B16 adhesion to HSE was investigated by using IL-18bp. We reported that hepatic metastasis of intrasplenically injected B16 cells was dramatically reduced in IL-1beta KO mice and almost completely inhibited in ICE KO mice [86]. Because ICE regulates the processing of both the IL-1beta as well as the IL-18 precursors into biologically active molecules, the ICE KO mice represented a quasi double knockout mutant.

We then further characterized the role of endogenous IL-18 in hepatic metastasis by blocking this cytokine using the naturally occurring IL-18bp [24]. A single intraperitoneal dose of IL-18bp given 30 min before intrasplenic injection of B16F10 cells reduced the number of hepatic metastatic foci by 75% and metastatic volume by 80%. Same treatment reduced the intrahepatic retention of luciferase-transfected B16 by 50% and abolished VCAM-1 up-regulation in the hepatic microvasculature, as assessed by RT-PCR, Western blot, and immunohistochemistry. Twelve hours after IL-18pb, HSE cells were isolated, and adhesion of B16 cells to these cultured HSE cells was reduced to the level of vehicle-treated mice. IL-18bp treatment of mice with established micrometastases resulted in a 25% decrease in metastasis number and 40% decrease in metastasis volume, suggesting inhibition of endogenous growth factors.

Assuming the inhibition of IFNgamma production by IL-18bp does not impact on the immune response to the malignant cells, IL-18bp may be useful as adjuvant therapy in reducing adhesion of malignant cells to vascular endothelia.

4.2 Role of tumor-derived IL-18 in cancer progression

Similar to other proinflammatory and proangiogenic cytokines, both human and rodent cancer cells can produce functionally competent IL-18. Some researches have focused on the mechanisms contributing to the regulation of IL-18 production by cancer cells. Others have analyzed the effects of IL-18 on cancer cell behavior.

Jiang et al. [87] compared the protein expression profiles between highly and poorly metastatic sublines, originating from the same parental human lung giant cell carcinoma PLA801 cell line. Compared with those in poorly metastatic subline, IL-18 was significantly upregulated in the highly metastatic subline. The association of IL-18 with metastasis was further elucidated by introducing IL-18 sense and antisense into low and highly metastatic PLA801 sublines. Their results demonstrated that ectopically expressed IL-18 promoted cell motility *in vitro* and down-regulated E-cadherin expression of low metastatic cell transfectants, while IL-18 antisense remarkably decreased cell invasion potency *in vitro* and notably increased E-cadherin expression of highly metastatic cell transfectants, indicating that IL-18 might play a role in enhancing tumor metastasis of lung cancer by down-regulating E-cadherin protein expression.

Park et al. [31] reported the expression of IL-18 by various human melanoma cell lines SK-MEL2, G-361, DM-4, and DX-3. Cho et al. [88] also demonstrated that highly metastatic B16F10 melanoma cells express IL-18. Interestingly, mouse C57BL/6 splenocytes cultured with B16F10 melanoma cell supernatant enhanced IFNgamma production, which was blocked by anti-IL-18 antibody, indicating that IL-18 in the culture supernatants was functional. In addition to IL-18, the IL-18R was also detected in these melanoma cells. The functional effect of IL-18 on B16F10 melanoma cells was shown by reduction of Fas ligand expression in cells treated with anti-IL-18 antibody or transfected with IL-18 antisense cDNA. The same treatments also decreased intracellular reactive oxygen intermediate levels in B16F10 melanoma cells, suggesting that IL-18 regulates reactive oxygen intermediate production, which is involved in Fas ligand expression. Furthermore, transfection of IL-18 antisense cDNA into melanoma cells increased the susceptibility of tumor cells to NK cells *in vitro*. When IL-18 antisense transfectants were implanted into syngeneic mice, severe reduction of tumor cell growth was observed with concomitant infiltrated NK cells in the tumor area. These results demonstrate that endogenous IL-18 induces immune escape by upregulating the Fas-L expression of melanoma cells, thereby down-regulating the susceptibility to host killer cells and therefore, acting as a survival factor for these melanoma cells. Next, Cho et al. [89], by using primary human epidermal keratinocytes and human keratinocyte cell line HaCaT, found that functionally active IL-18 production is enhanced by UVB irradiation in a dose- and time-dependent manner. The effect of UVB irradiation was blocked by antioxidant, *N*-acetyl-L-cysteine, which suggested the involvement of reactive oxygen intermediates in the signal transduction of UVB irradiation-enhanced IL-18 synthesis. They also found that AP-1 activation is required for UVB-induced IL-18 production. Interestingly, Hue et al. [90] reported that transfection of B16F10 melanoma cells with IL-18 antisense cDNA reduced stem cell factor expression—known

to be associated with melanocyte proliferation. Conversely addition of exogenous IL-18 to melanoma cells enhanced stem cell factor expression. Again, this effect was blocked by *N*-acetyl-L-cysteine and p38 mitogen-activated protein kinase inhibition, suggesting that IL-18 regulates stem cell factor expression via reactive oxygen intermediates and p38 MAPK activity.

To characterize the prometastatic balance of IL-18 in our experimental metastasis model of hepatic colonization of B16F10 melanoma cells, we have studied the direct action of endogenous (host cell derived) and exogenous (recombinant murine) IL-18 on the B16F10 melanoma cell adhesion to HSE cells *in vivo* and *in vitro* and analyzed involved cell adhesion molecules. HSE cells given B16F10 melanoma cell-conditioned medium significantly increased IL-18 over baseline level and subconfluently growing B16F10 cells given tumor-activated HSE-conditioned medium (HSE-CM) for 6 h significantly increased their adhesion to other HSE cells with respect to untreated HSE-CM-treated B16 cells. IL-18bp (1 μg/ml) abrogated the proadhesive action of B16F10 melanoma-treated HSE-CM on melanoma cells. Recombinant murine IL-18 produced a time- and dose-dependent proadhesive stimulation on subconfluently growing B16M cells *in vitro*, which reached the highest rates at 1 ng/ml for 6 h (three-fold over untreated cells). Anti-mouse VLA-4 (alpha4 integrin) antibodies 100% decreased IL-18-induced B16F10 melanoma cell adhesion augmentation. NFkappaB translocation also significantly increased in IL-18-treated B16F10 melanoma cells, as shown by EMSA, and either 1 μg/ml proteasome inhibitor MG-132 or 1,000 units/ml bovine catalase completely abrogated IL-18-induced adhesion augmentation in B16F10 melanoma cells. These results demonstrate that IL-18 upregulates VLA-4 activity in melanoma cells via oxidative stress-dependent NFkappaB translocation, which increases their adhesion to VCAM-1-expressing endothelial cells. These results also suggest that IL-18, either tumor-induced as endogenous factor or exogenously administrated, may facilitate intravascular arrest of circulating melanoma cells via synchronous cell adhesion molecule upregulation on both melanoma and endothelial cells [91] (Fig. 1).

Further confirmation on the regulatory role of the endogenous anti-oxidant machinery of melanoma cells in the regulation of functional effects of IL-18 on melanoma cells was obtained by simultaneously studying IL-18-induced adhesion in exponentially and subconfluently growing B16F10 melanoma cells. Again, IL-18 significantly increased the adhesion of subconfluently growing B16F10 melanoma cells to endothelium, but did not alter the adhesion rate of exponentially growing B16F10 melanoma cells. However, pre-incubation of exponentially growing cells with l-butionin-(*S*,*R*)-sulfoximin—a glutathione depletor—made it possible the proadhesive action of IL-18 on these melanoma cells. Conversely, the pre-incubation of subconfluently growing cells with *N*-acetyl-L-cysteine prevented the proadhesive action of IL-18 on these melanoma cells. These results uncover the important role played by the anti-oxidant machinery—herein represented by the cell growth-associated intracellular concentration of glutathione—in the modulation of cancer cell response to IL-18 [91]. On the other hand, addition of TNF-neutralizing antibody to IL-18-treated melanoma cells completely abrogated proadhesive effects of IL-18 while addition of IL-18bp to TNFalpha-treated melanoma cells did not alter the adhesion increase. This suggests that IL-18 operates in this melanoma model via TNFalpha. Moreover, IL-18 also induced TNFalpha expression in melanoma cells via oxidative stress-dependent mechanism, indicating once again that oxidative stress is a key mediator of multiple functional effects of IL-18 on melanoma cells [91].

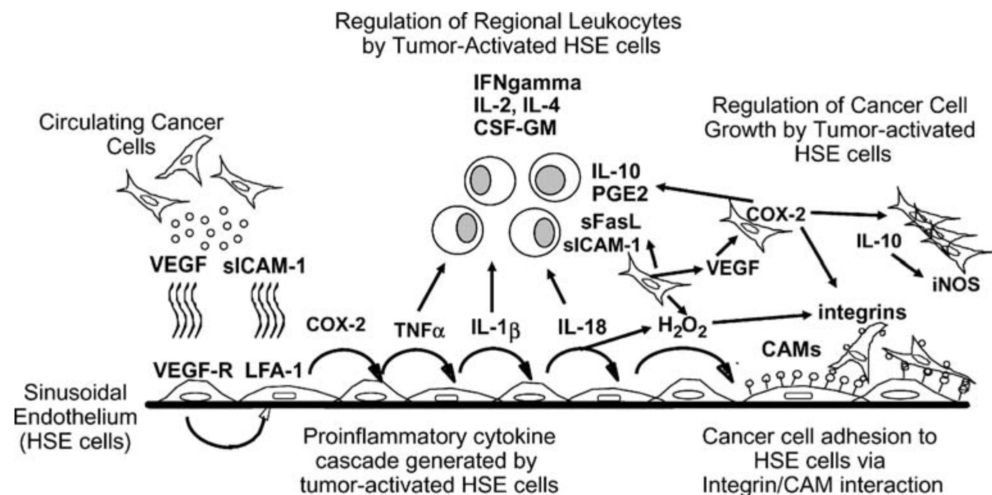

Fig. 1 Tumor-induced host cell production of IL-18 in the sinusoidal microenvironment of the liver. *HSE*, hepatic sinusoidal endothelium

In contrast to the evident proadhesive response of B16F10 melanoma to IL-18, around 15% B16F10 melanoma cell expressed IL-18R under basal culture conditions, as shown by flow cytometry using anti-mouse IL-18Ralpha antibodies. However, IL-18R gene expression significantly increased in IL-18-treated B16F10 melanoma cells, as shown by RT-PCR. Again, the mechanism was oxidative stress-dependent and increased the IL-18R-expressing melanoma cell fraction by two-fold. In turn, this shift into IL-18R-expressing cells occurred at the expense of those melanoma cells having a glutathione level threshold enabling them to express IL-18R in response to IL-18. This redox mechanism progressively enlarged the melanoma cell fraction able to respond to IL-18 that may account for the generalized cell responses observed in IL-18-treated cancer cells (Figs. 2 and 3).

Using gastric cancer cell lines, Majima et al. [92] found another mechanism of cancer cells that allows their immune escape and progression utilizing IL-18. In their model, all gastric cancer cell lines constitutively expressed IL-18 receptors and IL-18 dose-dependently enhanced their *in vitro* proliferation accompanied by NFkappaB activation. When IL-18-pretreated gastric cancer cells were cultured with cytokine-activated peripheral blood killer lymphocytes, the antitumor machineries, perforin or IFNgamma production of killer lymphocytes decreased, resulting in a decreased susceptibility of cancer cells to killer lymphocytes. Furthermore, gastric cancer cells cultured with IL-18 showed an increased expression of a granzyme B inhibitor, protease inhibitor 9. IL-18 injections into severe combined immuno-deficient mice intraperitoneally inoculated with gastric cancer cells consistently decreased the mouse survival time. These results suggest that gastric cancers may exploit IL-18 to grow and evade immunosurveillance. More importantly, these data are consistent with clinical studies reporting that higher IL-18 levels in sera and peritoneal lavage fluids in patients with gastric cancer correlated with an unfavorable outcome [22].

4.3 Proangiogenic effects of IL-18

Angiogenesis provides nutrient supply for cancer growth and a vascular access to cancer cells for their systemic dissemination. The balance between pro-angiogenic and anti-angiogenic factors is responsible for the presence and intensity of tumor angiogenesis. Angiogenic factors are produced by tumor cells and/or by tumor-infiltrating inflammatory cells, such as macrophages or polymorphonuclear leukocytes, and stromal cells, such as myofibroblasts. As above reviewed, several studies have reported on anti-angiogenic properties of IL-18. However, other studies in both neoplastic and non-neoplastic models have uncovered unexpected proangiogenis effects of both host- and tumor-derived IL-18. For example, Park et al. [93] reported that IL-18 induced human microvascular endothelial cell migration. Rheumatoid arthritis synovial fluids also potently induced endothelial cell migration, but IL-18 immunodepletion resulted in a significant decrease in migration. IL-18 appears to act on endothelial cells via alphaV-beta3 integrin. IL-18 also significantly increased endothelial cell tube formation on Matrigel matrix *in vitro*. IL-18 also induced angiogenic activity *in vivo*, as shown in IL-18-containing Matrigel plugs and sponges embedded with IL-18 implanted in mice. These findings supported a novel function for IL-18 as an angiogenic factor in rheumatoid arthritis and may elucidate a potential therapeutic target for angiogenesis-directed diseases. Consistent with these findings, Gerdes et al. [94] reported the expression of IL-18 and functional IL-18R on human vascular endothelial cells and smooth muscle cells. Qiao et al. [95] reported that IL-18 knockout mice showed angiectasis and vascular leakage, in a model of retinal vessel formation in IL-18 knockout and wild-type

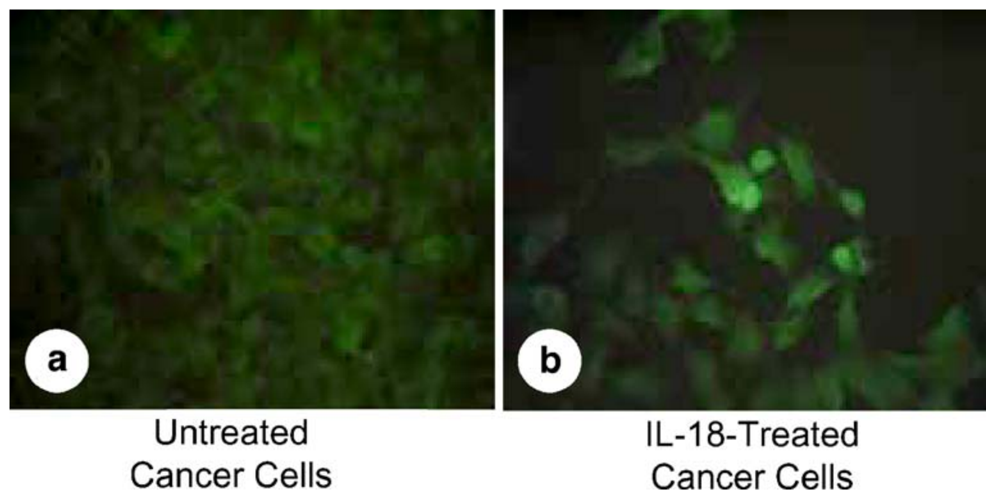

Fig. 2 Immunohistochemical staining of NFkappaB translocation in untreated (a) and IL-18-treated (b) B16 melanoma cells, as revealed using anti-p65 NF-kappaB antibody. IL-18-inducible cells are identified as small clusters of cancer cells with stained nuclei, representing a minority of the whole population

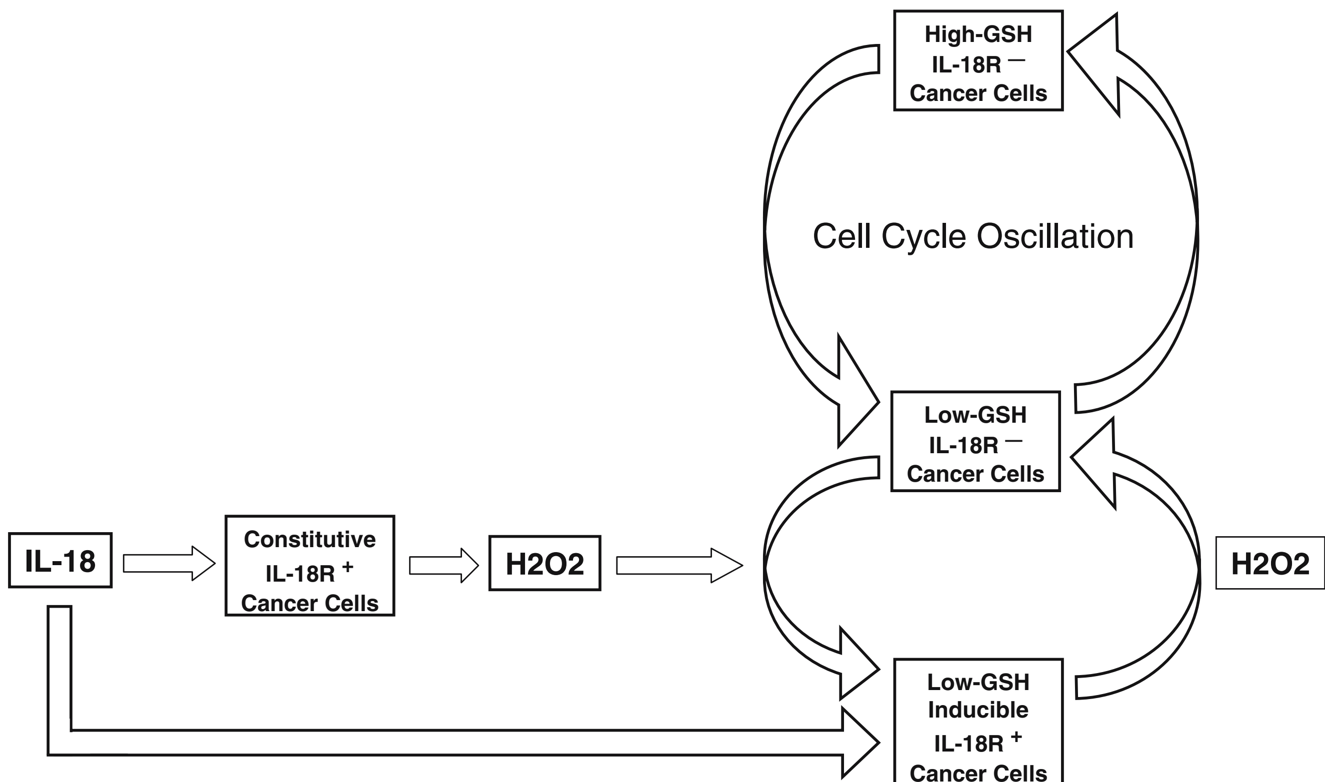

Fig. 3 Model of Microenvironment-induced IL-18 responsiveness in cancer cells via oxidative stress-dependent mechanism. *GSH*, glutathione; *IL-18R*, interleukin-18 receptor

mice. These symptoms were not observed in wild-type mice. Histopathological analysis confirmed abnormal vascular formation in IL-18 knockout mice. Several angiogenesis-associated factors, including VEGF, bFGF, PDGF and PEDF, were overexpressed in the retinas of IL-18 knockout mice compared with those of wild-type mice. IFNgamma was detected only in wild-type mouse retinas. These results provided new evidence for the role of IL-18 in vascular development. Cho et al. [96] found that IL-18 dose-dependently increased the production of VEGF production in fibroblast-like synoviocytes isolated from the patients with rheumatoid arthritis. The effect of IL-18 on VEGF production appeared to be as potent as IL-1beta, whereas TNFalpha and IFNgamma showed little effects on VEGF production. AP-1-specific inhibitor Curcumin dose-dependently abrogated the effect of IL-18 on VEGF production, suggesting IL-18 as an angiogenic factor in rheumatoid arthritis. In patients with cancer, Alexandrakis et al. [52] detected that increased serum IL-18 in myeloma patients correlated with advanced disease, increased levels of angiogenic cytokines and worse survival.

We recently [97] analyzed the role for endogenous IL-18 in hepatic stellate cells (HSC) and hepatic sinusoidal endothelium (HSE) cell recruitment into metastatic tissue *in vivo* by specifically blocking this cytokine using a soluble IL-18bp. In addition, the effects of IL-18bp on VEGF production along hepatic metastasis growth, on VEGF-dependent migration of cultured HSE and HSC, and on proliferation rate of metastatic tumor cells were also studied. VEGF (which is tumor and host-derived) increased during cancer cell colonization in the hepatic blood. IL-18bp significantly decreased (by 75%) both HSC and HSE cell recruitment in hepatic melanoma metastasis, leading to reduced angiogenesis and tumor growth. IL-18bp also significantly decreased VEGF concentration in the hepatic venous blood from metastasized livers until non-tumor-bearing mouse level, and it abolished VEGF production from B16 cells cultured either under hypoxic atmosphere or in the presence of HSE cell-conditioned media. Consistent with *in vivo* data, IL-18bp inhibited HSE cell and HSC migration induced by B16M-derived VEGF and recombinant murine VEGF *in vitro*. VEGF mRNA (RT-PCR) and protein production (ELISA) increased by twofold in 8-h-cultured B16 cells under 3% hypoxic atmosphere as compared to normoxic controls. IL-18bp abrogated hypoxia-induced VEGF transcription. Recombinant murine IL-18 (1 ng/ml, 6 h) increased VEGF via TNFalpha in cultured B16F10 cells. These results demonstrate that IL-18 mediates proangiogenic action of VEGF on myofibroblast and endothelial cell recruitment into hepatic melanoma metastasis, and the possible anti-metastatic therapy elicited by abrogating VEGF-depen-

dent HSC and HSE migration with IL-18 blockade. We already know that B16M cell-conditioned medium estimulates VEGF production from HSCs (hepatic miofibroblasts), which additionally contributes to the level of VEGF in the hepatic blood during cancer colonization. In summary, VEGF (which is tumor and host-derived) increases during cancer cell colonization in the hepatic blood and this may contribute to tumor neoangiogenesis. However, IL-18bp completely abrogated it. Thus, together with our knowledge on its inhibitory action of the proliferation-stimulating effect of host IL-18 on melanoma cells, IL-18bp may also inhibit VEGF-dependent angiogenesis. Both effects would explain the significant growth-inhibitory action of IL-18bp during late steps of B16 metastasis development in the liver [24].

We investigated effects of endostatin (ES) in the prometastatic microenvironment of inflammation occurring during the microvascular phase of cancer cell infiltration in the liver [25]. We used a model of intrasplenic injection of B16 melanoma cells leading to hepatic metastasis through VCAM-1-mediated capillary arrest of cancer cells via IL-18-dependent mechanism. We show that administration of 50 mg/kg recombinant human (rh) ES 30 min before B16, plus repetition of same dose for three additional days decreased metastasis number by 60%. A single dose of rhES before B16 injection reduced hepatic microvascular retention of luciferase-transfected B16 by 40% and inhibited hepatic production of TNFalpha and IL-18 and VCAM-1 expression by HSE cells. Consistent with these data, rhES inhibited VCAM-1- dependent B16 cell adhesion to primary cultured HSE receiving B16F10-conditioned medium, and it abolished the HSE cell production of TNFalpha and IL-18 induced by tumor-derived VEGF.

5 Conclusions

IL-18 plays a central role in inflammation and immune response, and is generally acknowledged as a key defense cytokine against infectious agents. Because immune-stimulating effects of IL-18 have also antineoplastic properties, it was tempting to propose IL-18 as a novel adjuvant therapy against cancer. However, recent results from clinical and experimental studies on the role of IL-18 in cancer progression have clearly demonstrated the multiple action mechanisms of this cytokine in the regulation of cancer cell and its microenvironment.

IL-18 concentration increases in the blood of the majority of cancer patients without being clear for the moment its functional impact. Frequently, augmentated IL-18 levels are directly associated to disease progression and aggressiveness, and correlate with tumor stage, clinical outcome and survival. Even more, in some cancer types the increase of IL-18 may indicate metastatic recurrence risk.

At present it is unclear whether pro- or anti-tumoral effects of IL-18, or both together, are operating in the pathophysiological complexity of cancer development. Some studies suggest cancer inhibition by IL-18 effects. Evidence on immune response-dependent antitumoral effects of IL-18 come from the enhanced antitumor response and improved survival obtained with direct administration of IL-18, alone or in combination with other cytokines (IL-2, IL-12) and miscellaneous agents (NO, 5-FU). Additional support comes from evidences on cancer progression when IL-18 responsiveness for IFN-gamma production is impaired; and when down-modulation of IL-18-induced immune response is produced by secreted IL-18 binding proteins encoded by infectious agents. Third, several studies have also suggested that antineoplastic effects of IL-18 may also occur via angiogenesis and osteoclastogenesis inhibition. In contrast, other studies reveal activation of cancer progression by IL-18 effects. Several mechanisms can operate in this way. First, tumor-induced endogenous IL-18 can promote experimental melanoma metastasis via cancer cell adhesion to microvascular endothelium and tumor growth-stimulating factor production. Second, IL-18 is produced as part of the functional proteome expressed by metastatic cancer cells. In this regard, multiple roles have been associated to tumor-derived IL-18, including: escape immune recognition via Fas ligand, E-cadherin down-regulation, VCAM-1 and VLA-4 integrin upregulation facilitating microvascular adherence of cancer cells, and production of VEGF, stem cell factor and other stromal and tumor growth-stimulating factors. Effects of IL-18 on host and cancer cells are usually mediated through the transcriptional activation via oxidative stress-induced NFkappaB. Furthermore, despite the low number of cells expressing IL-18R under basal conditions, production of reactive oxygen intermediates by IL-18-activated cells induce IL-18R expression, by low-glutathione content cells, which further amplify the responsiveness to IL-18.

Therefore, although IL-18 might be helpful for certain patients with cancer, blockage of IL-18 bioactivity by use of IL-18bp is also likely a promising therapeutic approach for other patients whenever risk of infectious complications can be clinically controlled. However, this review demonstrates that although several experimental models have uncovered possible mechanisms on the pro- and anti-tumoral effects of IL-18, our knowledge on the effects of IL-18 during human cancer development is still very limited and deserves further clinical investigation prior to consider IL-18 and IL-18bp as potential therapeutic agents againt cancer progression.

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