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CCL2 (monocyte chemoattractant protein-1) and cancer

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Abstract

Genetic analyses of cancer in humans indicate that chemokines and their receptors are unlikely to play direct roles in pathogenesis. However, these molecules have pleiotropic effects that impact on cancer pathobiology in animal models, and there is evidence that they may do the same in humans. Given their protean properties, chemokines could have tumor-promoting, tumor-suppressing activities, or either depending on context. An example is found in CCL2, a chemokine that attracts and activates mononuclear cells. In some settings, it stimulates host anti-tumor activities. However, tumor cells themselves secrete CCL2 suggesting that it has growth promoting effects. These have been documented in animal models and clinical epidemiological studies. If CCL2's protumorigenic activities can be validated, then CCL2 and its receptor CCR2 may be therapeutic targets in cancer.

Keywords: Breast cancer; CCL2; CCR2; Chemokine; MCP-1

1. Introduction

In essentially all settings, and regardless of tumor type, whenever evidence for chemokine or chemokine receptor expression in cancer has been sought, it has been found (see examples in [1]). Because of their ubiquitous expression and our detailed understanding of chemokine function in other settings, it has been tempting to infer that chemokines play an important role in tumor biology. However, this assumption should be viewed with a healthy skepticism. For example, one could argue from first principles that chemokines are rather unlikely to be centrally important in cancer pathophysiology. This argument would point out that the single most important insight into cancer pathogenesis to emerge from the past 25 years of research is that cancer is fundamentally a genetic disease. Wholesale gene loss, inactivation through interstitial deletion or mutation, overexpression through amplification or translocation, or viral mimicry of these processes underlie cancer pathogenesis. Furthermore, our understanding of basic processes that control cell proliferation has grown concomitantly so that we can see how most of these abnormalities produce neoplasia by their effects on growth factor receptor signal transduction, cell cycle checkpoint controls, or DNA synthesis. A fundamental validation of these insights is the fact that reverse genetics identifies these same genes as cancer susceptibility loci. However, to date, no susceptibility gene in any cancer has been mapped to a chemokine or chemokine receptor locus. The only example that even comes close is the increased incidence of cervical carcinoma in women with WHIM syndrome [2]. Although this disease is associated with CXCR4 mutations, its accompanying cervical cancer susceptibility reflects an immune deficiency that is permissive for high risk human papillomavirus infection rather than an inherent abnormality in cervical epithelial cell biology.

While this may be a valid argument against the idea that chemokines contribute to cancer pathogenesis, it does not rule out the possibility that they play an important role in cancer pathobiology. Research in cancer biology has demonstrated that, in addition to cell autonomous abnormalities in neoplasia, various host factors can have a profound effect on cancer behavior. These include host inflammatory states and immune deficiencies that reduce a postulated inherent resistance to some cancers. In fact, WHIM syndrome falls into this category. Because chemokines are involved in such a broad array of normal host activities that impact cancer, it is likely that they will have important effects on cancer pathobiology that may be amenable to therapeutic manipulation.

Just as chemokine effects in normal physiology are highly pleiotropic, their effects on cancer are likely to be multifaceted as well. Thus, chemokines might be expected to have either growth promoting or growth inhibiting influences on cancers depending on the particular setting in which they are expressed. For example, because of their ability to attract and

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activate leukocytes, some chemokines might be expected to stimulate host anti-tumor responses. Nonetheless, some of these same chemokines are known to have angiogenic activities which could actually contribute to tumor growth and progression. Furthermore, some chemokines can have direct effects on tumor cells. This review will consider data that support the notion that monocyte chemoattractant protein-1 (MCP-1, CCL2) is one of the chemokines that can have any or all of these effects on cancers.

2. Biology of CCL2 and CCR2

CCL2 (the systematic designation for MCP-1) is a potent chemoattractant for monocytes, memory T lymphocytes, and natural killer (NK) cells [3]. Although these properties were first defined on the basis of in vitro assays using purified proteins, subsequent experiments involving injected proteins in rodents or transgenic expression in specific organs demonstrated that these properties were faithfully reproduced in vivo [4–7]. CCL2 is structurally and genetically related to other chemokines (MCP-1, -2, -3, and -4 in humans, MCP-1, -2, -3, and -5 in the mouse) that share similar properties. The reason for their shared biological activities is that they all activate the same receptor, CCR2, with similar potencies. While the apparent promiscuity of CCR2 for multiple ligands was thought to imply a degree of redundancy in the MCP system, this has not turned out to be the case. Mice engineered to be deficient for CCL2 alone have unique phenotypes, most of which match those seen in CCR2-deficient mice [8–11]. The basis for specificity in vivo appears to lie in unique patterns of expression of the various MCPs in vivo. Thus, MCP-3 (CCL7), for example, does not substitute for CCL2 in CCL2 knockout mice because it is not expressed by the tissues that normally express CCL2. That is, when CCL2 is absent, the other MCPs are not upregulated to compensate for its absence [8]. This, of course, has therapeutic implications, namely that individual chemokines can be targeted for antagonism with some hope of success.

The unique effects of the CCL2/CCR2 axis on mononuclear cell migration suggested that it would be an important regulator of inflammatory disease. This prediction has been validated through the use of genetically deficient mice, antibody- or inhibitor-mediated neutralization in mice, and epidemiological studies in humans. One of the clearest examples is cardiovascular disease. Current models of the pathogenesis of atherosclerosis suggest that endothelial cells suffer damage as a result of persistently elevated levels of oxidized cholesterol as well as the shear stresses of hypertension or disordered blood flow [12]. In response, circulating monocytes are drawn into the subendothelium where they differentiate into macrophages and attempt to repair the damage. However, ongoing hypercholesterolemia leads to chronic cholesterol uptake by macrophages which become “stuck” in the arterial wall. Their chronic activation also leads to the secretion of smooth muscle cell chemoattractants and growth factors, resulting in the appearance of the mature atherosclerotic plaque. CCL2 was thought to be an excellent candidate for the monocyte recruitment signal secreted by injured endothelial cells. This was confirmed by analysis of CCL2- or CCR2-deficient mice. In several murine atherosclerosis models, absence of CCL2 or CCR2 substantially reduced arterial lipid deposition [13–16]. In all cases, amelioration of disease was associated with diminished numbers of macrophages in the arterial wall consistent with a model in which CCL2 contributes to atherosclerosis by attracting monocytes into the subendothelium via CCR2 activation. Similar results were observed in experimental allergic encephalomyelitis, which is a rodent model for multiple sclerosis [17,18]. Again, CCL2- or CCR2-deficient mice recruited many fewer monocytes into the site of disease (the central nervous system in this case), and the severity of disease was greatly reduced.

There are clinical epidemiological data to support the notion that CCL2 and CCR2 may play similar roles in human disease. Several studies indicate that increased plasma levels of CCL2 following balloon angioplasty of coronary arteries predicts for early restenosis, which in some ways can be thought of as accelerated atherosclerosis [19,20]. At a population level, a polymorphism in the CCL2 promoter has been demonstrated to be associated with an increased likelihood of an individual’s having coronary artery disease [21]. Interestingly, this polymorphism has been shown to produce increased rates of CCL2 transcription in vitro suggesting a mechanism for this association [22]. On the receptor side, a CCR2 polymorphism that responds less robustly to CCL2 in vitro compared to the more common CCR2 allele is associated with protection from coronary artery disease [23]. Thus, mouse modeling and human clinical investigation support the importance of CCL2 and CCR2 in inflammatory disease.

Other aspects of CCL2 and CCR2 biology are not so clear cut. Although the roles of this ligand and its receptor are concordant in inflammatory disease models, as revealed by their respective mouse mutants, the phenotypes of CCL2- and CCR2-deficient mice are quite distinct from each other in adaptive immunity models. Substantial evidence indicates that CCL2-deficient mice have defective type 2 immunity while CCR2-deficient mice have defective type 1 immunity [8,9,24,25]. The basis for this disparity is not yet clear, but it appears to involve effects on accessory cells, most likely those that are involved in antigen presentation. Although the mechanisms underlying these defects has not been fully elucidated, their potential contribution to CCL2/CCR2 effects in cancer must be kept in mind.

3. Anti-tumor effects of CCL2

Addition of CCL2 to macrophages in tissue culture enhances their anti-tumor activities [4]. This property plus CCL2’s ability to attract monocytes and macrophages in vivo has led to the not unreasonable suggestion that CCL2

could also have anti-tumor properties in vivo. In fact, animal modeling has demonstrated that CCL2 can suppress tumor growth both in T lymphocyte-independent and -dependent manners. In one example, malignant Chinese hamster ovary cells (CHO cells) were engineered to express varying levels of either human or murine CCL2 [26]. These cells, or their counterparts transduced with control expression vectors, were then injected into nude mice. Within 2 weeks, the control cells formed large tumors at the injection site. In contrast, CHO cells expressing high levels of either human or murine CCL2 formed no tumors. Notably, CHO cells expressing lower levels of CCL2 did form tumors but with a substantial delay in onset and growth rate, suggesting that CCL2 was exerting a dose-dependent anti-tumor effect.

To investigate the mechanism of CCL2's effect, the CHO cell injection site was examined histopathologically soon after inoculation. CCL2-expressing cells elicited a dense mononuclear cell infiltrate that was substantially greater than the infiltrate induced by control cells. Similar results have been observed in comparing the in vivo growth properties of cloned murine sarcoma cell lines derived from the same tumor that expressed different amounts of CCL2 [27]. As with the CHO cells, the sarcoma cells expressing high levels of CCL2 appeared later, grew more slowly, and were associated with a more intense monocyte/macrophage infiltrate. Thus, in at least a few different models, CCL2 can be shown to stimulate host anti-tumor responses in a T lymphocyte-independent manner.

Another potentially relevant consequence of CCL2's ability to stimulate mononuclear cells might be an enhancement of tumor antigen uptake or presentation. This was tested in a model designed to determine if CCL2 could increase the potency of a tumor vaccine [28]. Rat 9L glioma cells were rendered mitotically inactive by gamma irradiation and then injected subcutaneously in syngeneic rats in an attempt to stimulate specific anti-tumor immunity. Vaccine efficacy was tested by challenging vaccinated rats with 9L cells and monitoring the rate of tumor growth. When rats were vaccinated with irradiated but otherwise unmanipulated 9L cells, tumor growth by the injected challenge cells was slightly delayed compared to tumor growth in unvaccinated animals. This indicates that 9L cells are inherently somewhat immunogenic. However, rats vaccinated with 9L cells engineered to express CCL2 were completely protected from tumor growth after challenge injection with wild type 9L cells. Thus, in addition to stimulating T cell-independent anti-tumor effects in the models described above, CCL2 can also act as an adjuvant to enhance T cell-dependent host anti-tumor responses.

4. Protumor effects of CCL2

4.1. Biological and epidemiological considerations

Although the anti-tumor effects of CCL2 cited above are experimentally convincing, these are unlikely to be CCL2's primary physiologic influences on cancer behavior. As noted in the introduction, many cancers express chemokines and CCL2, in particular, appears to be expressed by a wide variety of cancer types. In fact, one of the original discoverers of CCL2 called it tumor-derived chemotactic factor because of its secretion by several tumor cell lines [29]. However, CCL2's frequent expression in cancer is somewhat paradoxical when considered in the context of its anti-tumor activities. Cancers are highly Darwinian systems. One of the molecular hallmarks of a cancer cell is its genetic instability which results in an accelerated mutation rate. The selective pressures for autonomous proliferation are enormous and lead to the appearance of cells that have acquired mutations endowing them with growth factor independence and the capacity to elicit their own vasculature. Conversely, mutations that inactivate pathways irrelevant for tumor growth are also advantageous because their loss redirects energy stores toward the business of proliferation. Hence, the so-called dedifferentiation of cancers that arise from highly specialized tissues (although this is likely to be a misconception [30]). It would seem counterproductive, if not downright anti-Darwinian, for biologically successful cancers to persist in the production of chemokines like CCL2 if their primary function were to assist the host in destroying the tumor.

A more likely reason for cancer cells to secrete chemokines is that they actually contribute to tumor growth and progression. This could occur in different ways. Because many cancer cells express functional chemokine receptors, chemokines might directly stimulate their proliferation or survival. This has been observed in glioblastoma cell lines in which CXCR4 activation by SDF-1 (CXCL12) provides an antiapoptotic signal [31]. Alternatively, tumor-derived chemokines might act indirectly by attracting mononuclear cells to the tumor site where they could provide growth or angiogenic factors that would enhance tumor progression. This is a concept that originated with Paul Ehrlich [32], but has been given a contemporary and rigorous basis by Mantovani et al. [33].

Regardless of mechanism, these considerations suggest that CCL2 expression might be advantageous for the tumor and disadvantageous for the host. Support for this idea can be found in a Japanese study of 135 breast cancer patients who had the CCL2 content of their tumors measured by analysis of biopsy material [34]. Patients were stratified into those with high or low levels of tumor-associated CCL2 based on whether tumor CCL2 concentrations were above or below the median for the study population. After nearly 6 years of follow-up, the women with high levels of tumor-associated CCL2 had a significantly shorter relapse-free survival.

Interestingly, CCL2 levels also correlated with the abundance of tumor-associated macrophages. This observation places CCL2 expression into the larger context of pathological and epidemiological studies that have identified tumor macrophage content as an adverse prognostic indicator in breast cancer and raises the possibility that CCL2 is pri-

marily responsible for the recruitment of tumor-associated macrophages in this disease [35–37]. A similar set of observations has been made in ovarian, gastric, and esophageal carcinomas [38–40]. As internally consistent as this idea might be, however, it should be kept in mind that there is very little direct evidence supporting it, and an alternative explanation might involve direct effects of CCL2 on mammary carcinoma cells. For example, mammary carcinomas cell lines have been shown to undergo chemotaxis in response to CCL2 [41]. Thus, it remains possible that macrophage content is an epiphenomenon.

4.2. CCL2 and angiogenesis

Tumor-induced vessel formation has been recognized since the early 1900s and, more recently, has been exploited as a therapeutic target in cancer. In addition to potent, well validated angiogenic factors such as VEGF, chemokines have also been ascribed angiogenic properties. Most attention has been focused on CXC chemokines that contain the glutamate–leucine–arginine (ELR) N-terminal motif [42], but CCL2 has also been suggested to have proangiogenic activity [43]. This is a complex issue because of CCL2's proinflammatory activity. The macrophages that CCL2 attracts are potent sources of other angiogenic factors, including VEGF, and there is some question about whether CCL2's effects on angiogenesis are direct or whether they are exerted through macrophage recruitment and activation [44]. For example, the increased vascularity associated with CCL2-secreting tumors [39,40] also correlates with their macrophage content. While CCL2 or CCR2 blockade might prevent tumor angiogenesis, the effect could be an indirect one.

Nonetheless, microvascular endothelial cells express CCR2 and respond chemotactically to CCL2 [43]. Furthermore, CCL2 has been reported to have angiogenic activity both in the chorioallantoic membrane assay and in vivo in a manner that is independent of inflammatory cell infiltration [43]. Administration of anti-CCL2 antibodies to mice carrying breast carcinoma xenografts prolongs their survival (although this may not necessarily occur through inhibition of angiogenesis (see below)) [43]. Thus, it remains possible that CCL2 could have a direct tumor angiogenic effect although it must be noted that neither CCL2- nor CCR2-deficient mice have an abnormal angiogenic phenotype.

4.3. CCL2 and direct effects on tumor cells

Because of the large amount of information already available about the effects of chemokines on leukocytes, much of the modeling of chemokine effects in cancer is based on the assumption that they exert their effects via leukocyte recruitment or activation. However, it is quite clear that chemokine receptor expression is not limited to hematopoietic cells, and there have been several reports describing the repertoire of chemokine receptors displayed by a variety of tumor cell types [45–47]. In some cases, relevant functional information is also available. The best known example involves CXCR4 which has been suggested to be involved in patterns of target organ involvement during metastatic spread [48]. Perhaps even more relevant is the observation that CXCL12 activation of CXCR4 in glioblastoma cells provides an antiapoptotic signal that could lead to tumor cell survival and enhanced overall tumor growth [31].

By analogy, it is possible that CCL2 could also have direct effects on tumor cell physiology. In fact, CCR2 expression has been documented in various tumor types, including breast cancers, and some breast cancer cell lines respond chemotactically to CCL2 in vitro [41]. Although chemotactic migration in vitro should not be considered as a surrogate for malignant behavior in vivo, this observation does indicate that some breast cancer cells respond directly to CCL2. In preliminary studies, we have observed that CCL2-deficient mice are protected in models of endogenous mammary carcinoma development, consistent with the anti-CCL2 antibody studies cited above. So far, we have not observed an obvious decrease in the intensity or quality of the leukocytic infiltrate associated with these tumors, and so the possibility remains that CCL2 could be exerting a direct effect on mammary carcinoma proliferation or survival.

5. Summary

Evidence from clinical epidemiology, histopathology, preclinical animal modeling, and in vitro tissue culture studies

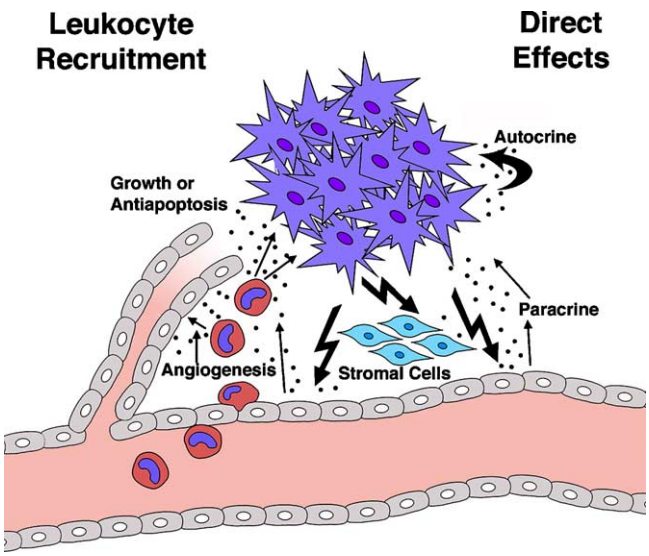

Fig. 1. CCL2 could promote tumor progression in two ways. The effects of tumor-derived CCL2 could be indirect, as depicted on the left, through its recruitment of leukocytes that provide growth or antiapoptotic signals to tumor cells, or that provide angiogenic signals. Tumor-derived CCL2 could also be angiogenic. Conversely, CCL2 could provide direct pro-tumor effects as shown on the right: tumor-derived CCL2 could act in an autocrine fashion, or tumor cells could stimulate stroma or endothelium to secrete CCL2 (jagged arrows) that would impact tumor cells in a paracrine manner.

all point to the possible involvement of CCL2 and CCR2 in cancer pathobiology. Unfortunately, there are insufficient data to permit a mechanistic understanding of what this chemokine ligand/receptor pair is actually doing in cancer. The two major possibilities are presented in broad outline in Fig. 1. In both cases, CCL2 might be secreted by tumor cells themselves, or tumor cells might stimulate CCL2 production by nearby stromal or endothelial cells. On one hand, this CCL2 might exert its effects by recruiting mononuclear cells as depicted on the left side of the figure. These cells could secrete growth or survival factors that would enhance tumor progression, or they could provide angiogenic factors such as VEGF that could stimulate vessel formation and also lead to tumor growth. On the other hand, CCL2 could act in a paracrine or autocrine manner to promote tumor cell growth or survival. Considerable work will be required to establish whether either or both of these mechanisms are relevant to authentic tumor pathobiology. If CCL2's effects in cancer can be validated, then it may be reasonable to consider targeting CCL2 or CCR2 in this disease.

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