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Inflammatory chemokines in cancer growth and progression

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ABSTRACT

Leukocyte infiltration is a cardinal feature of almost all cancers. Chemokines are generally responsible for eliciting local accumulation of inflammatory cells and they appear to play the same role in the formation of peri- and intra-tumoural infiltrates. Chronic inflammation predisposes to cancer formation and progression, and it is likely that the chemokine system contributes to this process. In part, this may be a consequence of its ability to attract mononuclear cells to cancer sites, where they provide growth or angiogenic factors that enhance cancer development. However, accumulating evidence also points to a direct effect of chemokines on cancer cells that express chemokine receptors. In particular, some chemokines can activate anti-apoptotic pathways in these cells. By either mechanism, tumour cells that secrete and/or respond to chemokines would have a selective advantage. This provides another example of cancer's ability to co-opt host systems in order to promote tumour progression.

1. Introduction

For well over 100 years, pathologists have recognised that almost all cancers are accompanied by inflammatory cells. Thoughts about the function or 'purpose' of this infiltrate have evolved over time as biologists have gained a deeper understanding of basic mechanisms of inflammation. In the 19th century, peri-tumoural inflammatory cells were thought to be an integral part of cancer.[1,2] A little later, their presence was proposed to provide a favourable environment for cancer growth.[3–5] This included the now contemporary notion that inflammatory cells might elaborate signals that promote development of new blood vessels, which are necessary for cancer progression. Still later, as experimental evidence for host anti-tumour immune responses accumulated, infiltrating cells were suggested to be evidence for the host's attempt at immunological rejection of its cancer.[6–8] Most recently, the pendulum has swung back to suggestions that tumours might recruit inflammatory cells because of their ability to provide signals that promote cancer growth.[9]

Understanding the molecular mechanisms that generate tumour-associated inflammatory infiltrates might help to illuminate their function. Among the predominant signals that recruit leukocytes in all inflammatory settings are chemokines. These are low molecular weight proteins that share a high degree of structural homology and the ability to attract specific types of leukocytes with picomolar potencies. More than 45 non-allelic chemokine genes have been identified in the human genome, and their combinatorial interactions with more than 20 chemokine receptors account for the variety and specificity of leukocyte types found in various inflammatory infiltrates.[10] The almost universal presence of chemokines in cancers suggests that in this setting, too, the chemokine system is responsible for specific patterns of leukocyte accumulation.

However, even if there is general agreement that chemokines attract leukocytes to tumours, the function of these elicited leukocytes (and, by extension, the function of chemokines themselves) remains controversial. This review will summarise the experimental evidence that chemokines can

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affect the behaviour of cancers and that they and their receptors might therefore be exploitable therapeutic targets.

2. Chemokines and tumour immunity

Chemokines are potent attractants for cells involved in innate immune responses. Many chemokines can also induce activation programs in target leukocytes, some of which may be relevant to the host's response to cancer. For example, in vitro studies have shown that macrophage-attracting chemokines, such as monocyte chemoattractant protein-1 (MCP-1, CCL2) also stimulate macrophage cytolytic activity against tumour cells.[11] Given sufficient activation, these cells might have non-specific anti-tumour activity in vivo. This has been demonstrated in animal models, although their artificiality may somewhat limit the pathophysiological relevance of the findings. Nonetheless, a typical example is the observation that engineered overexpression of CCL2 in tumour cells elicits a strong mononuclear cell infiltrate when these cells are injected in vivo, even in immune deficient mice, and leads to prompt tumour rejection.[12,13] Similar experimental approaches have been used to evaluate the anti-tumour properties of a large number of other chemokines (Table 1). As in the CCL2 example, most of these experiments involve transduction of genes encoding chemokines of interest into a variety of tumour cell types followed by grafting in immune-deficient or immune-competent hosts. However, in some instances, direct injection into the tumour site of recombinant chemokines (e.g., CCL1[14] or CCL21[15]) or viral vectors encoding chemokines (e.g., CCL27[16] or CCL16[17] or CCL21[18]) can be effective (see Fig. 1).

Table 1 – Chemokines with anti-tumour activities[a]

Primary rejection	References	Memory response	References
CCL1	14	CCL3	26,76,77
CCL2	12,13	CCL16	17,78
CCL3	76,79,80	CCL19	81,82
CCL4	83	CCL20	76,84
CCL5	85,86	CCL21	18,30,87,88
CCL7	89,90	CCL22	91
CCL16	17,78	CCL27	16,30
CCL19[b]	15,81,82,92,93	CXCL9	94
CCL20[b]	76,84	CXCL10	95
CCL21	18,87,96		
CCL22	91		
CCL27	16,30		
CXCL2	35		
CXCL4	97,98		
CXCL8[c]	35,36		
CXCL9	94,95,99,100		
CXCL10	95		
CXCL14	101		
XCL1	102		

a Chemokine activities are separated into direct effects, i.e., those that lead to tumour rejection when injected or engineered to be expressed by tumour cells, and memory effects, i.e., those that enhance tumour rejection upon rechallenge.
b But see Ref. [103].
c But see Ref. [31–34].

In addition to these innate effects, some chemokines can also affect adaptive immunity by attracting cells involved in this arm of the immune response. On the afferent side, so-called immune chemokines, such as CCL20, attract immature dendritic cells to inflammatory sites via activation of the chemokine receptor, CCR6.[19] Just as this is thought to be an early step in the acquisition of foreign antigens for later presentation, CCL20 might similarly attract these cells to tumour sites where they could take up and process tumour antigens. Activation of these cells by Toll-like receptor (TLR) ligands or cytokines is then thought to induce the expression of specific chemokine receptors in these cells, which stimulate their migration to secondary lymphoid organs where they have the potential to initiate an immune response (assuming tolerance has been abrogated).[20–22]

This effect could be exploited in the physiologically artificial but therapeutically important setting of tumour vaccination, where it might be expected that these chemokines would have adjuvant properties. For example, when tumour cells engineered to over-express CCL2 are injected into syngeneic animals, a T cell-dependent anti-tumour response can be elicited that provides substantial protection for the host against subsequent challenge with the same tumour cells.[23] While the mechanism underlying this effect is not known, there is a suggestion that CCL2 might induce dendritic cell migration to draining lymph nodes.[24] Although the source of that CCL2 might be localised within the node, CCL2 at the tumour site may be translocated to lymph nodes by a mechanism involving lymphatic transport.[25] Similar enhancement of specific anti-tumour immunity can be achieved using several other chemokines (Table 1). On the efferent side, chemokines might attract effector cells back to the tumour site. For example, adenoviral transfer of CCL3 to established tumours in mice enhances the infiltration into the tumour of adoptively transferred, tumour-specific T lymphocytes.[26]

Given the fact that such a large number of chemokines have anti-tumour activity, especially in the innate models, it seems difficult to ascribe any specificity to this property. One explanation for this near-universal efficacy may be that it reflects the network-like nature of soluble inflammatory mediators. Once a mediator initiates an inflammatory infiltrate, the cell type that first appears is often capable of secreting other mediators that attract other effector cells. This eventually results in a common terminal outcome: activated mononuclear cells engaging in tumour cell lysis. This might explain why almost all CC and some CXC chemokine subgroups are active in these systems. However, a subset of CXC chemokines is an obvious exception to this rule (e.g., CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7 and CXCL8). These are the chemokines that have the three amino acid motif, glutamate-leucine-arginine (ELR), in their N-terminal domains, a motif that is specific for interacting with the chemokine receptors CXCR1 and CXCR2.[27,28] It has been suggested that these chemokines are angiogenic in vivo, and that this might explain their pro-tumourigenic activity.[29] Conversely, the anti-tumour activity of several ELR-negative CXC chemokines (e.g., CXCL9, CXCL10 and CXCL11) may be based on their anti-angiogenic properties. Along the same lines, reports of anti-tumour activity of CX3CL1[30] have been contradicted by other reports that this chemokine lacks any

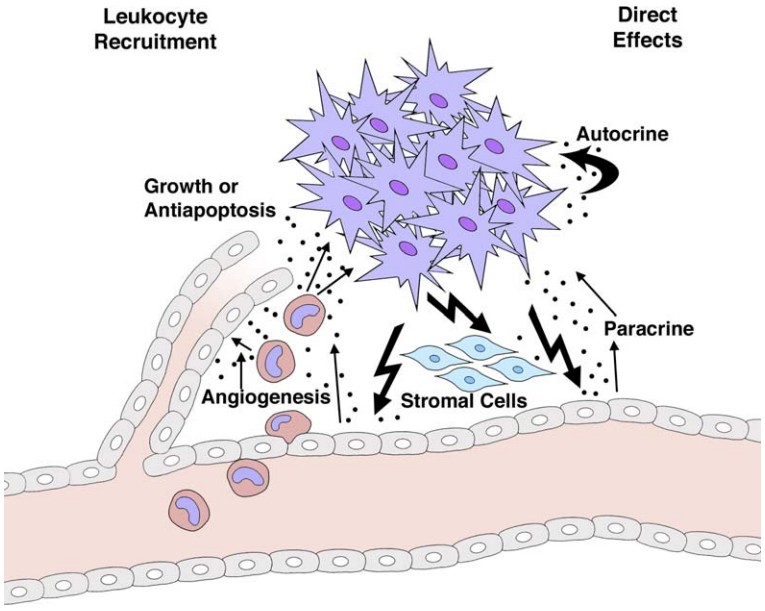

Fig. 1 – Dual role for chemokines in cancer progression. Tumour cells and surrounding stroma may secrete chemokines in order to attract leukocytes (usually mononuclear cells) that provide growth or angiogenic factors that can stimulate cancer progression (left). However, these chemokines can also have direct effects on tumour cells that express their cognate receptors (right). In particular, activation of chemokine receptors might promote proliferation or inhibit apoptosis. Either way, inflammation will enhance tumour growth. Reprinted with permission.

activity in these settings because its anti-angiogenic properties counteract whatever innate immune function it may have.[16] It is important to note that these chemokines may exert their anti- and pro-angiogenic effects directly on endothelial cells, that is they may not be dependent on leukocyte recruitment. However, the anti-tumour leukocyte recruitment and pro-tumour angiogenic effects may not be mutually exclusive. For example, even though IL-8 is a paradigm ELR-positive CXC chemokine, and even though its expression in tumour cells can promote their growth in vivo by enhancing angiogenesis,[31–34] overexpression of IL-8 in lung cancer models prevents metastasis presumably through neutrophil recruitment.[35,36] Similarly, engineering cells to express low levels of CCL2 can enhance tumour growth.[12] Although this may also be a reflection of CCL2's angiogenic activity,[37] other mechanisms may be relevant (see below).

Despite these caveats, the overall impression given by Table 1 is that chemokines as a class generally have anti-cancer activity. While these effects are quite clear and have the potential to be exploited for therapy, they are not likely to be relevant in helping us understand why almost every cancer cell that has been examined secretes chemokines. Cancers are highly Darwinian systems in which genomic instability produces frequent mutations and epigenetic effects produce heritable modulations in gene expression.[38] This is an ideal setting for natural selection to exert its influence. For example, a tumour cell that loses a differentiated function that does not contribute to its growth will have a substantial selective advantage over tumour cells that are 'wasting energy' by retaining that function. Thus, by the time a tumour is clinically detectable, the cells comprising it are not likely to be expending energy in pathways that do not contribute in some way to the tumour's biological success. Even if the effect of tumour-derived chemokines were indifferent with respect to tumour growth, there would still be selective pressure to eliminate chemokine synthesis. It is that much less likely, then, that biologically successful tumours would secrete chemokines if they were involved in host anti-tumour activities. Rather, the fact that so many biologically successful tumours secrete chemokines, and not just ELR-positive CXC chemokines, strongly suggests that they must contribute to tumour growth or progression.

3. Indirect mechanisms: tumour-associated leukocytes and stromal cells as delivery devices

One possible mechanism is an indirect one that was first proposed by Ehrlich[3] and has recently been brought up-to-date and enhanced by Mantovani, Balkwill, Pollard and others.[9,39] Theirs is the notion that tumours secrete chemokines or other leukocyte attractants in order to elicit leukocytes that provide growth factors for cancer cells or angiogenic factors for the tumour-associated neovasculature. Clinical epidemiological evidence supports this idea. For example, in breast cancer, several studies have demonstrated that increased numbers of tumour-associated macrophages portends a poor clinical outcome.[40–42] At least one report has tied this observation directly to CCL2. A small study of women with early stage breast carcinoma demonstrated that those whose tumours expressed high levels of CCL2 had increased numbers of tumour-associated macrophages and a significantly shorter relapse-free survival.[43] Similarly, levels of CCL2 expression in breast tumours correlate with their histological stage.[44]

However, none of these studies demonstrates rigorously that infiltrating macrophages directly stimulate tumour cell proliferation or tumour angiogenesis. To be sure, increased

numbers of tumour-associated macrophages correlate with increased neovessel density,[45] but no causal relationship has been demonstrated either clinically or in animal models. Nonetheless, many of the molecular elements that would be consistent with this model are present in tumour infiltrates. Macrophages are sources of potent angiogenic factors, such as vascular endothelial growth factor (VEGF), as well as less potent but possibly relevant factors such as interleukin-8 (IL-8) and other ELR-positive CXC chemokines. Of course, some tumour cells themselves secrete these chemokines and to the extent that these proteins may be pathophysiologically relevant tumour angiogenic factors, they may also contribute to tumour progression. Along the same lines, there is evidence that CCL2 itself may also be angiogenic.[37] These observations were based on several conventional assays, e.g., chorioallantoic membrane and Matrigel plug assays, which included a potentially confounding inflammatory infiltrate that might have provided other, authentic angiogenic factors. However, similar effects were observed in isolated aortic rings in which, it has been suggested, inflammatory responses are not likely to occur.

In addition to leukocytes, stromal fibroblasts can also deliver inflammatory chemokines that affect tumour behaviour. Recent data suggest that stroma-derived SDF-1 (CXCL12) acting through its sole receptor CXCR4 can promote tumour progression by stimulating angiogenesis.[46] Specifically, carcinoma-associated fibroblasts derived from mammary carcinomas secrete CXCL12, which attracts endothelial progenitor cells to tumour sites. Myofibroblasts from normal mammary tissue do not have the same supportive properties.

These observations are consistent with a model in which tumours that secrete chemokines themselves, or induce fixed phenotypic alterations in stromal cells that result in chemokine secretion, have a selective advantage either because they attract leukocytes or because they enhance angiogenesis. In both cases the peri-tumoural cells that are either attracted to the tumour site or are affected by nearby tumour growth act as delivery devices for growth factors or angiogenic factors that promote tumour progression.

4. Direct mechanisms: chemokines may promote tumour growth

Another way that inflammatory chemokines could promote tumour growth would be through direct action on tumour cells themselves. This would require the expression of functional chemokine receptors by malignant cells. It is not surprising, perhaps, that haematopoietically derived cancer cells express a variety of chemokine receptors. The stem cell population from which they derive normally gives rise to cells that use chemokine receptors for their physiological functions, suggesting that the malignant counterparts might retain this expression pattern if it provided a selective advantage.

However, epithelial malignancies also express chemokine receptors, although their function is not clear. There are data suggesting that tumour cell migration, i.e., patterns of metastatic spread, might be regulated by chemokine ligand/receptor interactions.[47] Again, this function is easily understood in haematopoietic malignancies because the chemokine system drives migratory behaviour of their normal progenitors. For example, chronic lymphocytic leukaemia and lymphoma cells that undergo widespread dissemination express CXCR5 and CCR7, receptors that are involved in normal B and T lymphocyte trafficking.[48–52] But, it has been suggested that malignant cells derived from epithelial precursors may have the same property. In particular, clinical patterns of breast cancer metastasis have been suggested to be a consequence, in part, of CXCR4 expression and chemotactic response to its ligand.[47] Of course, an alternative explanation might be that micrometastases occur in a disseminated fashion, independent of chemokine receptor expression, and that macrometastases appear when locally expressed chemokine ligands stimulate proliferation or survival of tumour cells expressing the appropriate receptor.[53]

In fact, accumulating evidence suggests that chemokines may contribute directly to survival pathways in malignancy. For example, glioblastomas upregulate CXCR4 and their survival is prolonged in the presence of SDF-1.[54] In another example, the effect of CCL2 on breast cancer has been examined based on the clinical epidemiological evidence, cited above, that CCL2 expression is an adverse prognostic factor in this disease. A genetic model has been constructed in which mammary carcinoma-prone mice were placed in a CCL2-deficient background. *CCL2*$^{-/-}$ mice showed a delay in tumour appearance, decreased growth rate of tumours, and longer overall survival compared to *CCL2*$^{+/+}$ mice (I. Conti and B.J. Rollins, Dana-Farber Cancer Institute). Heterozygous *CCL2*$^{+/-}$ mice had an intermediate phenotype. These observations would be consistent with the clinical association between CCL2 expression and breast cancer outcome.[43] However, the tumour-associated infiltrate in the murine model was not diminished in the CCL2-deficient animals. Instead, CCL2's pro-tumourigenic mechanism might be based on autocrine secretion and activation of CCR2 on the tumour cells themselves, which inhibits apoptosis.

Interestingly, reports of anti-apoptotic effects of chemokines are appearing with increasing frequency. IL-8 counteracts apoptotic signals in B-cell chronic lymphocytic leukaemia[55] and ovarian carcinoma;[56] CXCL12 inhibits apoptosis in several different cell types including glioblastoma,[54,57] pancreatic adenocarcinoma,[58] B-cell chronic lymphocytic leukaemia[59] and small cell lung cancer;[60] CCL25 inhibits apoptosis in T cell acute and chronic lymphocytic leukaemia cells;[61] and CCL1 inhibits apoptosis in T cell acute lymphocytic leukaemia cells.[62,63] The variety of ligands and cell types involved in the anti-apoptotic response suggests that this may be a somewhat general effect of chemokines on malignant cells that express relevant receptors.

5. Genetic considerations

The contemporary 'gold standard' for implicating specific molecular involvement in cancer is demonstrating a genetic basis for that molecule's effect. Translocations and point mutations pointed to the essential contribution of BCR/ABL in chronic myelogenous leukaemia and the epidermal growth factor receptor in a subset of lung cancers. Of course, the ultimate confirmation of these observations has been the efficacy of imatinib and gefitinib in these diseases. To date, no genetic

abnormalities in chemokines or their receptors have been associated with a specific cancer or cancer predisposition, arguing, perhaps, that the chemokine system may not contribute significantly in a cell-autonomous manner to cancer risk.

However, differential representation of chemokine ligand or receptor polymorphisms in patients with cancer have been described. Some of these polymorphisms have functional consequences and may be relevant to cancer risk. For example, a well-characterised polymorphism in CCR2, the CCL2 receptor, occurs at position 64. The less frequent allele, 64I, is associated with decreased severity of coronary artery disease[64,65] suggesting that it may be less efficient in transducing inflammatory signals since CCL2 and CCR2 are required for full manifestations of atherosclerosis.[66–68] This same allele is underrepresented in patients with breast cancer and invasive cervical cancer.[69,70] Along the same lines, a polymorphism in the CCL2 promoter has been characterised in which the less frequent allele is associated with increased mRNA transcription.[71] Not surprisingly, this allele is associated with increased severity of atherosclerosis,[64] and this allele is also over-represented in patients with metastatic breast cancer.[72] Both of these observations are consistent with the clinical and mouse model data indicating the effect of CCL2/CCR2 signalling on breast cancer progression. Other chemokine polymorphisms have skewed representations in cancer, including CXCL12 alleles in breast cancer[73] lung cancer,[74] leukaemias and lymphomas.[75] These large, population-based genetic studies lend strong support to the notion that the chemokine system influences cancer appearance and behaviour.

6. Conclusion

Despite demonstrations of the anti-tumour effects of chemokines in artificial models, the preponderance of evidence – clinical, genetic and experimental – indicates that the chemokine system contributes to cancer progression. This represents one molecular facet of the general pro-tumourigenic effects of inflammation. Some of the involved mechanisms are straightforward, such as recruitment to the tumour site of leukocytes that provide angiogenic factors. Other mechanisms are more surprising but make sense. The anti-apoptotic effect of chemokines on malignant cells recapitulates their effect on leukocytes. Prolonging the survival of ordinarily short-lived cells, such as neutrophils, is one of the mechanisms that chemokines can use to enhance the inflammatory response. A tumour cell that exploits this anti-apoptotic signal transduction pathway will have a selective advantage when placed in an inflammatory environment with its soup of ambient chemokines. Fortunately, this pathway can be exploited therapeutically. Blockade of chemokine signalling might enhance apoptosis and produce a clinical response. But, because resistance to cytotoxic chemotherapy manifests as a resistance to apoptosis, blockade of chemokine receptor signalling would be an attractive approach to combine with conventional chemotherapy in order to increase its efficacy.

Conflict of interest statement

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