

Chemokines as Biomarkers

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Introduction

Chemokines have been defined as small cytokines involved in the migration and activation of cells such as lymphocytes and phagocytic cells, playing a central role in inflammation [1]. The term itself is a combination of chemotaxis and cytokine. Chemokines work through a large family of G protein coupled receptors. Chemokines are classified in families (CXC, C, CX3C, and CC) with the ligands named with an L and the receptors named with an R. For example IL-8 is classified as CXCL8 and its receptors are CXCR1 and CXCR2 [2]. However many chemokines are still known by their traditional names. In addition to inflammation, chemokines are major regulators of malignancy and are produced by tumor cells[3]. All cells are likely able to produce chemokines under certain conditions. Chemokines are redundant in their action on target cells and chemokine/receptor interactions are promiscuous with receptors interacting with multiple ligands [4]. Chemokines are present in the human plasma proteome at higher concentrations than cytokines [5] making them easier to detect and quantify using ELISA and mass spectroscopy techniques. Because of the widespread production, the stability and long half life of the proteins [6],and the higher concentrations at which they can be found, chemokines are being considered as biomarkers of various diseases. Serum CCL11 or eotaxin-1 has been shown to be present at concentrations in the hundreds of pg/ml [7] has been put forward as a biomarker for prostate cancer [8]. The interferon regulated chemokines have been shown to be effective as serum biomarkers in measuring the progression and activity in systemic lupus erythematosus. [9]. Urinary chemokines look promising as biomarkers of kidney damage such as lupus nephritis [10] and urinary Gro- α (CXCL1) looks promising as an early biomarker of renal ischemia/reperfusion [11]. Chemokines have also been examined as biomarkers of cardiovascular risk [12] with additional studies recommended to work out issues with sample collection and treatment as well as prospective studies [6]. Below is a brief discussion of the functions and roles of a few important cytokines as potential biomarkers of disease.

Eotaxin (CCL11)

Eotaxin is an eosinophil selective chemotractant with minimal effect on other leukocytes and its activity in vivo is elevated by the presence of IL-5[13]. It exerts its activity through the CCR-3. While CCR-3 is promiscuous, being activated by regulated on activation, normal T-cell expressed and secreted (RANTES) and MCP-2-4, eotaxin is the only chemokine to exclusively use CCR-3 [13]. Serum eotaxin (CCL11) along with interferon inducible protein-10 (IP-10) have been advanced as early biomarkers of age related macular degeneration [14],Eotaxin is constitutively expressed in the gut . In the airway eotaxin mRNA has been found to be produced in as little as 60 min following exposure of TNF- α , IL-1, or interferon gamma. Eosinophils constitutively express eotaxin wich may lead to autoamplifications of eosinophil recruitment into inflammatory sites. [13] Eotaxin also induces respiratory burst or the production of reactive oxygen species in eosinophils via phosphoinositide-3 (PI3K), protein kinase C (PKC) and

tyrosine kinases via inhibition studies [15] Eotaxin promotes the release of the anti-inflammatory cytokine IL-4 from eosinophils in a non-cytolytic manner that is enhanced by IL-5, but inhibited by brefeldin A an inhibitor of vesicle formation. It has been hypothesized that one of the mechanisms of glucocorticoid inhibition is through eotaxin suppression [15]. Eotaxin activity is controlled by cleavage and degradation by the membrane associated serine protease CD26 [15]. Elevated plasma eotaxin levels have been observed in subjects with liver cirrhosis and has been put forward as a biomarker for aging and cancer [16] as well as a biomarker indicating adverse prognosis in chronic liver disease [17]. Eotaxin ranges in that study were from 8.3-1578 and averaged 47.8 pg/ml in 111 patients with chronic liver disease[17].

GRO- α (CXCL1)

Originally thought to be a melanoma growth stimulatory factor and labeled melanoma growth stimulatory activity Gro- α is a chemokine that is structurally similar to interleukin (IL)-8 and is an activator of neutrophils inducing, chemotaxis, exocytosis, and respiratory burst [18]. Gro- α has been shown to be upregulated following administration of IL-2 to patients with renal cell cancer [19] and high concentrations of Gro- α predict radiographic progression in rheumatoid arthritis patients taking methotrexate [20]. As Gro- α is tumorigenic on nude mice as well as exhibiting the aforementioned stimulation of melanoma, it has been labeled as “the most striking evidence of the direct regulation of tumors and tumor growth by chemokines [21].” Gro- α is also commonly known as keratinocyte derived chemokines orKC.

I-309 (CCL1)

I-309 is a monocyte chemotractant protein that exerts potent anti-apoptotic activity [22]. It appears to be metastatic and has been shown to be significantly elevated in metastasis derived cell lines [23]. I-309, in conjunction with other proteins, has promise as a cerebrospinal fluid biomarker of Alzheimer’s disease progression and cognitive impairment; [24]. CCL1 may also be a biomarker of cardiovascular risk [25].

IL-8 (CXCL8)

IL-8 is a widely studied proinflammatory chemokine that is upregulated by NF- κ B signaling and exerts its effects via the cell surface receptors CXCR1 and CXCR2 [26]. Neutrophils appear to be major targets of IL-8 signaling. IL-8 has shown to be a promising biomarker for urinary bladder cancer, prostatitis, pyelonephritis, vesicoureteral reflux, osteomyelitis, inflammatory bowel disease, chorioamnionitis, non-Hodgkin’s lymphoma, and pulmonary and nasocomial infection [27]. IL-8 may also be an effective biomarker for acute kidney injury after liver transplant surgery [28], squamous cells carcinomas of the mouth [29], and intrathecal inflammation [30]. As IL-8 appears to be a potential biomarker in multiple diseases that are seemingly only related by the inflammatory process IL-8 exhibits a lack of specificity as a

biomarker pertaining to individual diseases. However, by multiplexing IL-8 with other disease associated biomarkers and performing network analysis [31] the presence of IL-8 can become a powerful biomarker for disease determination.

IP-10 (CXCL10)

Known as the interferon- γ inducible protein 10 or CXCL10, IP-10 is secreted by fibroblast, monocytes, and endothelial cells that have been stimulated by interferon- γ and it interacts with the CXCR3 receptor. IP-10 has been shown to down-regulate angiogenesis and promote T-cell migration to inflammatory sites. It has been put forward as a serum biomarker of systemic lupus erythematosus and lupus nephritis as there have been observations of increases in serum concentrations of IP-10 in patients diagnosed with the diseases [32]. IP-10 is a promising biomarker for Mycobacterium tuberculosis infection [33] as well as for asthma [34] and COPD [35] exacerbation following rhinovirus infection. CXCL10 is a potential biomarker for sepsis [36], severity of liver disease [37], as serum biomarker of viral infection [38].

MCP-1 (CCL2)

Monocyte chemoattractant protein (MCP-1 or CCL2) is a widely studied chemokine that is very potent in its ability to mobilize monocytes. Like many chemokines it is produced by multiple cell types such as smooth muscle, mesangial, astrocytic, microglial, fibroblasts, and epithelial, cells although the major producers of MCP-1 appear to be monocytes/macrophages. CCL2 is also thought to be an intervention point for treatment of many diseases [39]. MCP-1 has not only been shown to stimulate IL-4 production and attract monocytes to the site of inflammation contributing to their maturation into macrophages, it has been shown to be correlated with viral load in HIV infection [39]. It may have some antitumor activity [39] but it is also considered a pivotal mediator in the pathogenesis of atherosclerosis [40]. Urinary MCP-1 may be useful as a biomarker of SLE activity [41] while plasma MCP-1 has been postulated to be a marker of cardiac aging [42].

MCP-2 (CCL8)

MCP-2, like MCP-1, MCP-3 and MCP-4 binds to the CCR2 receptor [43]. Like MCP-1, MCP-2 has been found in multiple sclerosis brain lesions [44]. MCP-2 can be differentiated from MCP-1 in that MCP-2 stimulates eosinophils and basophils while MCP-1 activates only basophils [45]. In conjunction with IP-10, MCP-2 appears to be a biomarker for tuberculosis diagnosis [46] and possibly SLE [47] and rheumatoid arthritis [48].

RANTES (CCL5)

RANTES like MCP-1 attracts monocytes, and target memory T cells, (via different receptors) RANTES also triggers chemotaxis and activation of eosinophils [39]. It appears to be an

effective biomarker in differentiating infection from types of pneumonia [49] as well as other viral infections [50] and cardiac mortality [51].

TARC (CCL17)

Thymus and activation related chemokine (TARC) is produced by dendritic and other antigen presenting cells and attracts T cells via the CCR4. TARC appears to be a good marker for monitoring treatment and disease progression in patients with Hodgkin's disease [52]. It is a postulated target of therapeutics for allergies [53] and potential biomarker for atopic dermatitis [54]. It has also been investigated as a biomarker in diverse illness such as malaria [55], Gaucher's [56], and cancer [57].

Conclusion

While the characteristics of chemokines that make them easy to detect and quantify also limit the specificity of individual chemokines as biomarkers. As with many protein biomarkers, multiplexing appears superior to single protein[58]. It would likely be advantageous to consider examining alterations in chemokine concentrations in your research as well. The Quansys Human Chemokine array contains ELISAs for Eotaxin, GROa, I-309, IL-8, IP-10, MCP-1, MCP-2, RANTES, TARC which will allow you to measure alterations in the concentrations of the above mentioned chemokines. Also the chemokines can be readily combined with other cytokines such as interleukins for a low cost custom Q-Plex ELISA array delivered to you with minimal turnaround time.

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