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Human glioblastoma tumours and neural cancer stem cells express the chemokine CX3CL1 and its receptor CX3CR1

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ABSTRACT

Human gliomas represent an unmet clinical challenge as nearly two-thirds of them are highly malignant lesions with fast progression, resistance to treatment and poor prognosis. The most severe form, the glioblastoma multiforme, is characterised by a marked and diffuse infiltration through the normal brain parenchyma. Given the multiple effects of chemokines on tumour progression, aim of this study was to analyse the expression of the chemokine CX3CL1 and of its specific receptor CX3CR1 in 36 human surgical glioma samples, with different degrees of histological malignancy and in glioblastoma-derived neurospheres. Herein we show that both ligand and receptor are expressed at the mRNA and protein levels in most specimens (31/36). While receptor expression was similarly detected in low or high grade tumours, the uppermost scores of CX3CL1 were found in grades III–IV tumours: oligodendrogliomas, anaplastic astrocytomas and glioblastomas. Accordingly, the expression of CX3CL1 was inversely correlated with patient overall survival ($p = 0.01$). Glioblastoma-derived neurospheres, containing a mixed population of stem and progenitor cells, were positive for both CX3CR1 and for the membrane-bound chemokine, which was further up-regulated and secreted after TNF-IFNγ stimulation. Confocal microscopy of 3D neurospheres showed that the ligand was primarily expressed in the outer layer cells, with points of co-localisation with CX3CR1, indicating that this ligand–receptor pair may have important intercellular adhesive functions. The high expression of CXC3L1 in the most severe forms of gliomas suggests the involvement of this chemokine and its receptor in the malignant behaviour of these tumours.

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1. Introduction

Glioblastoma multiforme (GBM) is an aggressive tumour of the central nervous system, with high propensity to infiltrate throughout the brain, rendering it the most lethal primary brain tumour.[1,2] Despite different treatment modalities, 2-year survival is less than 30% and these results have remained unchanged over the last two decades.[2,3]

This poor prognosis has several causes: (i) glioblastomas are characterised by extensive dissemination of tumour cells within the normal brain areas, which renders total surgical resection virtually impossible without extensive neurological damage; (ii) these tumours are highly refractory to available chemotherapeutic and radiation treatments; (iii) clinicians and scientists have an insufficient understanding of the complex and aggressive nature of glioblastomas.

Established experimental evidence has demonstrated that tumour progression not only depends on the capacity of autonomous cell proliferation but also largely depends on external cues from the micro-environment.[4,5] In the reactive tumour stroma, now defined as inflammatory micro-environment, several growth factors, cytokines and proteolytic enzymes are expressed. Produced both by infiltrating macrophages, fibroblasts and tumour cells themselves, these mediators actively promote the angiogenic switch, tumour cell survival, proliferation and invasion of adjacent tissues.[6,7] Among the factors that contribute to the invasive phenotype of glioma cells, increasing attention has been directed to the chemokine system.

Several different chemokines are produced in the tumour milieu and constitute a paradigm of the cancer-related inflammation.[7–10] These chemotactic cytokines have a complex connection with tumour development. Chemokines were mostly studied for their potent effect on the recruitment of leucocytes at sites of inflammation or neoplasia; however, in the last decade it has become increasingly clear that tumours express also chemokine receptors and therefore neoplastic cells have the ability to mediate ligand-induced biological effects. It is now established that migrating malignant cells may exploit chemokine receptors to invade surrounding tissues and give distant metastasis.[7,8,11] In addition to cell mobilisation, chemokines enhance tumour cell resistance to apoptosis and/or proliferation and modulate angiogenesis and extra-cellular matrix turnover.[8,12–14]

Since one of the main features of GBM is its infiltrating potential[5] and given that several chemokines are produced in damaged brain areas to recruit immune cells and neural stem cells (NSCs), the present study aims to shed light on the putative role of the chemokine system in GBM progression. A number of studies have investigated the expression of chemokine receptors in gliomas. mRNA and protein expression for some specific receptors, like for instance CXCR3, CXCR4 and CXCL8 receptors has been reported.[15–17] In particular, the presence of CXCR4 has been associated with the most aggressive forms of gliomas and poor patient survival.[16,18,19]

Like other solid tumours, glioblastomas contain a small fraction of cells recognised as cancer stem cells (CSCs) or tumour-initiating cells (TIC), responsible for tumour origin, progression and recurrence *in vivo*.[20,21] A recent paper demonstrated that glioblastomas CSC growing as neurospheres highly express CXCR4 and this expression decreases in differentiated cells; moreover the stimulation with the specific ligand CXCL12 induces a significant proliferative response in CSC but not in corresponding differentiated cells.[19] These results suggest an important functional role of chemokines in cancer stem/progenitor cells.

One chemokine/receptor pair that has been rather poorly studied in tumours is CX3CL1 and CX3CR1. CX3CL1 was cloned from activated endothelial cells and neurons and originally termed Fractalkine or Neurotactin, respectively.[22,23] Unlike other chemokines, CX3CL1 is a transmembrane protein that can function as an adhesion molecule as well as a chemokine when cleaved by specific proteases. CX3CL1 is expressed in the nervous system mainly by neurons and astrocytes/glial cells, in inflammatory conditions.[24] CX3CL1 binds exclusively CX3CR1, a G-protein coupled receptor expressed mainly by leucocytes, including microglia in the brain.[25] Experimental studies highlighted the role of CX3CL1 in attenuating inflammation in the brain, thus indicating that the CX3CR1/CX3CL1 axis is a major player in the cross-talk between neurons and microglia, possibly contributing to the maintenance of homeostasis in the brain.[26–30] Only recently the receptor CX3CR1 was investigated in human malignancies and found to be expressed by cancer cells of prostatic and pancreatic carcinoma, and involved in tumour spread.[31,32] CX3CR1 was recently reported in established tumour cell lines originated from GBM,[33,34] in human glioma and in tumour-infiltrating leucocytes.[35,36]

Herein we show that both ligand and receptor are expressed in human glioma surgical samples, and that levels of CX3CL1 significantly correlated with severity of disease and inversely with overall survival. Glioblastoma-derived neurospheres, containing a mixed population of stem and progenitor cells, also express CX3CR1 and CX3CL1 and confocal microscopy images suggest that this axis is an important adhesive loop.

2. Materials and methods

2.1. *Patients and tissues specimen*

Tumours were collected from a series composed by 36 patients, 22 men and 14 females. All patients were operated by craniotomy and removal of the tumour at the Dept. of Neurosurgery, IRCCS Istituto Clinico Humanitas. Patients who were simply biopsied were excluded from the study. The histopathological diagnosis was made by a single pathologist (PC) and subsequently reviewed by a consultant neuropathologist. The lesions were classified along the WHO classification. In order to verify the differences in CX3CL1 and CX3CR1 expression in different oncotypes we analysed 8 cases of slowly growing tumours (3 cases of grade I astrocytomas and 5 cases of grade I and II oligodendrogliomas) and 28 cases of malignant tumours (4 oligodendrogliomas grade III, 2 cases of anaplastic astrocytomas grade III and 22 cases of glioblastoma grade IV). The overall survival, age of patients and tumour localisation were evaluated. All the patients gave their informed consent.