CHARACTERIZATION OF CHEMOKINES AND THEIR RECEPTORS IN THE CNS: PHYSIOPATHOLOGICAL IMPLICATIONS

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Chemokines are chemotactic proteins that regulate the trafficking and circulation of the leukocyte population across blood, lymph and tissues, in particular during immune response. They also regulate development, homeostasis, and function of immune system cells, and are involved in the regulation of angiogenesis. In recent years it has become evident that chemokines also exert important functions in the CNS, as testified by their widespread expression, both constitutive and inducible, in neurons and glial cells. The involvement of a chemokine in brain development has been demonstrated by studies on knockout mice SDF-$1^{-/-}$ or $CXCR4^{-/-}$, that showed serious defects in cerebellar development, characterized by the disruption of the regular laminar architecture, probably due to a disorganized migration of precursor cells. Recent studies show that some chemokines act as neuromodulators by their ability to influence the synaptic activity either through the stimulation of neurotransmitter release or by regulating postsynaptic receptor activity through second messengers. Moreover, chemokines are involved in CNS pathologies like multiple sclerosis, Alzheimer's disease, ischemia, and AIDS dementia, where anomalous levels of several chemokines have been observed. Chemokines could also have a role in brain tumor progression: they could be involved in the metastasis process, by determining the target organ of metastatic cells; moreover IL-8 and SDF-1 are supposed to act also as mitogens for malignant cells. Indeed the expression of the SDF-1 receptor CXCR4 is up-regulated in human glioblastoma cell lines, and correlates with the malignancy of the tumor grade in human gliobastoma tissues.
We studied the expression and the role of chemokines and their receptors in healthy brain cells as and in malignant transformed glia. Our studies revealed that the chemokine receptors CXCR4 and CCR5 are widely expressed in the adult rat brain. We detected by RT-PCR, northern blot, and immunocytochemistry the expression of the chemokine receptor CXCR4 in cultured rat type I astrocytes, cerebellar granule cells and cortical neurons. We demonstrated that its ligand SDF-1á is expressed, at mRNA level, by the same cells, and that is also secreted constitutively by cerebellar granules and in response to LPS stimulation by astrocytes. Microfluorimetric studies showed that SDF-1á is able to induce the release of intracellular calcium in astrocytes and cortical neurons, that was completely abolished, in astrocytes, by treatment with pertussis toxin. We also have investigated the involvement of SDF-1á in the control of astrocyte proliferation and the intracellular pathways that mediate this process. Our results indicate, that SDF-1 induces a dose-dependent astrocyte proliferation, as well as the activation of the MAP kinase ERK1/2. Both cell proliferation and ERK1/2 activation were reduced by PD98059 (an inhibitor of MEK), wortmannin (an inhibitor PI-3K), and by pertussis toxin. In the SDF-1á signaling, PI-3K is necessary not only for the ERK1/2 activation, but also for Akt, as we demonstrated in western blot by the use of wortmannin. We also identified in the protein tyrosine kinase Pyk2 another downstream effector. We studied the expression of CXCR4 and SDF-1 in several human glioblastoma tumor tissues and in human glioblastoma cell lines (DBTRG-05MG, U87-MG, and U373). Our data showed that CXCR4 was expressed in all tissues analyzed while SDF-1 was expressed only in some tumors. On the contrary the cell lines express both CXCR4 and SDF-1. These cells are able to secrete SDF-1α in basal conditions and the rate of secretion is increased by LPS. Our preliminary data indicate that stimulation by exogenous SDF-1α induces cell proliferation in two cell lines, phosphorylation of ERK1/2, both inhibited by PD98059, and Akt activation, indicating the involvement of ERK1/2 and, perhaps, of Akt in SDF-1–induced glioblastoma cell proliferation. Furthermore, we demonstrated the ability of a monoclonal anti-hCXCR4 antibody, clone 12G5, to inhibit the SDF-1α–induced proliferation. This antibody is also able to block the increase of cell proliferation induced in the presence of 1% FCS, indicating that SDF-1α could act as an autocrine regulator of glioblastoma growth in vitro.
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