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Colony Stimulating Factor-1 Expression in Human Glioma

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ABSTRACT

Colony stimulating factor 1 (CSF-1) is a functionally versatile, circulating homodimeric growth factor that stimulates the survival, proliferation, and differentiation of mononuclear phagocytic cells, the differentiation of osteoclast progenitor cells and that regulates cells of the female reproductive tract. CSF-1 is also expressed in the central nervous system where it may regulate the differentiation and activation of microglia. The diverse forms of CSF-1 are all encoded by a single gene. Alternative posttranscriptional splicing and posttranslational cleavage determines whether CSF-1 will be produced as a secreted proteoglycan, secreted glycoprotein, or as a cell-surface glycoprotein that may be involved in cell–cell interactions. CSF-1 is expressed in glioblastoma cell-lines, normal human astrocytes, and in operative specimens of human glioma. The CSF-1 receptor, encoded by the c-fms proto-oncogene, is also expressed in human gliomas. We conclude that coexpression of CSF-1 and its receptor in some human gliomas hints at a possible autocrine or paracrine growth stimulatory role for CSF-1; however, its function in the mammalian CNS remains to be elucidated.

Index Entries: CSF-1; c-fms; brain tumors; glioblastoma; growth factor; microglia; proteoglycan; glycoprotein; astrocytes.

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INTRODUCTION

Glioblastoma multiforme (GBM) is a highly malignant neoplasm that resists conventional therapies. Infiltration of the brain parenchyma, profound vascularity, and cellular heterogeneity all account for GBM's particularly malignant nature and its poor response to treatment. Cytoreductive surgery and external beam irradiation yield a median life expectancy of only 9–12 m from the time of diagnosis (Salcman, 1990). Brachytherapy, adjuvant chemotherapy, and numerous experimental interventions have failed to provide more promising clinical outcomes, leading many to conclude that improved therapies for glioblastoma will only result from a better understanding of cellular transformation in general as well as the complex biology of this particular neoplasm.

The study of polypeptide growth factors and their signal transduction pathways is essential to this end. Growth factors are the molecular effectors of primitive paracrine growth regulatory systems (Cross and Dexter, 1991) and in some cases, like endocrine hormones, travel to distant cellular targets via the circulation. Growth factor responsive cells possess transmembrane receptors that are activated by growth factor binding, initiating a cascade of intracellular events that lead either to cellular growth and proliferation, or differentiation.

Aberrations in this tightly controlled growth regulatory system may play a role in neoplastic growth. Support for this hypothesis is derived from the study of retroviral oncogenes (Bishop, 1987). During the course of a retroviral infection, segments of the host's DNA may be incorporated into the viral genome. Upon subsequent infections, this host gene is introduced into other cells and expressed by the viral transcriptional promotor. If the transduced gene encodes a protein that is involved in cell-cycle regulation, neoplastic growth may ensue. Any segment of host DNA may be retrovirally transduced, but only those genes that regulate growth and differentiation have the potential to function as oncogenes. In many instances, viral oncogenes have been demonstrated to be homologous to cellular genes that encode a growth factor or growth factor receptor. Although no human cancer has been definitively linked to a transforming retrovirus, the identification of growth factor-related genes as viral oncogenes has fostered great interest in the role polypeptide growth factors themselves play in the pathogenesis of human neoplasms as well as normal growth and development.

A number of growth factors have been implicated in the pathogenesis of human glioma (Cockram, 1990); however, no single abnormality has been consistently detected. Amplification of the epidermal growth factor receptor (EGFR) gene is the most frequently described growth factor-related aberration associated with GBM; yet, this abnormality is detected in only ~40% of glioma specimens studied (Wong et al., 1987). Other growth factor-related abnormalities occur less frequently, raising doubts

as to the significance of these findings. The large number of growth factors that have been detected in glioblastoma and the inconsistency with which they are found indicate that GBM is biologically as well as histologically heterogeneous, composed of multiple cellular subpopulations, each employing a distinct set of growth stimulatory systems.

Growth factors may also mediate more than one biological process during neoplastic growth. Basic fibroblast growth factor (bFGF) provides one such example. bFGF has been shown to be both an autocrine stimulator of glioma growth in vitro (Morrison et al., 1990) and a potent angiogenesis factor (Folkman and Klagsbrun, 1987). A recent study demonstrated a correlation between bFGF levels in a series of gliomas with the vascularity and the histologic degree of malignancy of the tumors (Takahashi et al., 1992). bFGF may therefore fulfill two critical roles in glioma progression, stimulating the proliferation of malignant astrocytes and promoting neovascularity.

Colony stimulating factor 1 (CSF-1) is an acidic glycoprotein or proteoglycan that regulates at least three important biological functions (Das et al., Stanley et al., 1983; Stanley, 1990). Initially described as a cytokine that selectively stimulates the survival, proliferation, and maturation of mononuclear phagocytic cells. CSF-1 was later found to play a critical role in the regulation of various cell types in the female reproductive tract during pregnancy (Pollard et al., 1987, 1991; Arceci et al., 1989; Stanley, 1990) and in the differentiation of osteoclast progenitor cells (Wiktor-Jedrzejczak et al., 1990; Yoshida et al., 1990; Felix et al., 1990). Preliminary evidence suggests that CSF-1 may regulate microglial differentation during neural development (Hao et al., 1990) and may also mediate inflammatory responses within the central nervous system (CNS) (Hulkower et al., 1991; Shafit-Zagardo et al., 1991; Thery et al., 1992). The following is an overview of CSF-1 biology focusing on its function in the central nervous system. We present preliminary evidence that CSF-1 is produced by transformed human astrocytes and discuss the role CSF-1 may play in the pathogenesis of malignant glioma.

CSF-1 BIOSYNTHESIS

The mouse and human genomes possess a single CSF-1 gene (Kawasaki et al., 1985) that is approximately 21 kilobases (kb) in length and is composed of 10 exons (Ladner et al., 1987). Exon 1 and part of exon 2 encode a 32 amino acid signal peptide that is characteristic of secreted proteins. Several different CSF-1 messenger RNA species encoding biologically functional forms of CSF-1 exist because of alternative splicing. Exons 9 and 10 encode alternately used 3′ untranslated sequences that probably confer varied stabilities on the mRNAs to which they belong (Ladner et al., 1987). Alternative splicing within exon 6 determines the

form of CSF-1 produced (Ladner et al., 1987). The region of exon 6 that can be spliced out of the primary transcript contains several proteolytic cleavage sites. Message that contains all of exon 6 encodes a precursor protein that possesses all of the proteolytic cleavage sites and a transmembrane domain toward the carboxyl terminus that anchors the protein within the endoplasmic reticulum and golgi apparatus (reviewed in Stanley, 1990). This precursor protein is cotranslationally glycosylated and rapidly dimerizes in the endoplasmic reticulum. Proteolytic cleavage in the secretory vesicle releases the dimeric form of the protein that is subsequently secreted on fusion of the vesicle with the plasma membrane. Depending on the site of proteolytic cleavage, the secreted growth factor will either be a glycoprotein or a chondroitin sulfate containing proteoglycan (Price et al., 1992). Splicing out of most of exon 6 produces mRNA species that encode proteins from which the proteolytic sites and glycosaminoglycan addition sites have been deleted. Consequently, these glycoproteins are expressed as plasma membrane spanning glycoproteins (reviewed in Stanley, 1990; Price et al., 1992) that are biologically active at the cell surface (Stein et al., 1990). Thus, CSF-1 can be expressed in three dimeric forms: a secreted glycoprotein, a secreted proteoglycan, and a cell-surface glycoprotein.

CSF-1 is detected at biologically active concentrations in the human circulation in both the glycoprotein and proteoglycan forms. The proteoglycan form may allow CSF-1 to interact with certain extracellular matrices, localizing the growth factor to specific cellular targets both locally and at a great distance from the site of CSF-1 production. The transmembrane form of CSF-1 may function at the cell-surface, mediating cell–cell interactions.

CSF-1 SIGNAL TRANSDUCTION

Within 10–12 h of CSF-1 stimulation, quiescent macrophages enter the cell-cycle and initiate DNA synthesis (Tushinski and Stanley, 1985). This effect of CSF-1 is mediated by a single class of high affinity CSF-1 binding sites (Guilbert and Stanley, 1980, 1986). The CSF-1 receptor (CSF-1R) is a 165 kDa transmembrane protein that possesses intrinsic tyrosine kinase activity (Yeung et al., 1987) and is encoded by the c-fms protooncogene (Sherr et al., 1985). The CSF-1R belongs to the family of "immunoglobulin-like" receptor tyrosine kinases which includes the platelet derived growth factor receptor (PDGFR), the bFGF receptor, and the c-kit protooncogene product (Ullrich and Schlessinger, 1990). CSF-1 binding leads to receptor dimerization, autophosphorylation, and other receptor modifications (Li and Stanley, 1991). In addition, a number of intracellular proteins are tyrosine phosphorylated in response to CSF-1 (Downing et al., 1988; Sengupta et al., 1988). Studies are currently underway to identify the protein substrates of the CSF-1R tyrosine kinase.

Characterization of these proteins, and study of the genes that encode them should lead to a more complete understanding of the signal transduction pathways through which CSF-1 elicits its biological responses.

CSF-1 AND THE CENTRAL NERVOUS SYSTEM

Recent studies indicate that CSF-1 may be involved in normal neural development and the regulation of inflammatory responses within the CNS. CSF-1 transcripts are detected in the adult mouse neocortex and in fetal mouse brains as early as the 14th d of gestation (Thery et al. 1990). Cultured mouse astroglia secrete CSF-1 but do not express the CSF-1R. In contrast, "microglia-like" cells derived from primary mouse brain cultures do express the CSF-1R and respond mitogenically to astroglial-derived CSF-1 (Hao et al., 1990).

Lewis rats with experimental autoimmune encephalomyelitis (EAE), an experimental model for multiple sclerosis, exhibit a rise in CSF-1 mRNA levels within the spinal cord 1–2 d prior to histologic and clinical evidence of an acute transverse myelitis. Remission is preceded by a decrease in CSF-1 gene expression (Hulkower et al., 1991). In vitro studies of murine astrocytes demonstrate that CSF-1 expression is enhanced by interleukin 1 and tumor necrosis factor, both potent cytokines (Shafit-Zagardo, 1991; Thery et al., 1992) Taken together, these data suggest that astroglia produce CSF-1 during neural development and acute inflammatory responses. During development, CSF-1 may serve to recruit circulating monocytes into the fetal nervous system, generating the resident microglial population. Alternatively, astroglial production of CSF-1 may stimulate the differentiation of neuroepithelial precursors along microglial lines. In the adult brain, CSF-1 may play the role of an immune-modulator, functioning as a chemoattractant for circulating monocytes and/or stimulating expansion of the resident microglial population.

CSF-1 IN HUMAN MALIGNANCY

CSF-1 has been implicated in the pathogenesis of a variety of human malignancies including Hodgkin (Paietta et al., 1990) and non-Hodgkin lymphomas (Nakamura et al., 1989), leukemias (Rambaldi et al., 1988), and carcinomas of the lung, pancreas, breast, and ovaries (Wu and Yunis, 1980; Kawasaki et al., 1985; Horiguchi et al., 1988; Kacinski et al., 1988, 1989a, 1991). Patients with myeloproliferative disorders, myeloid leukemias, and ovarian carcinomas exhibit elevated serum CSF-1 levels when compared to age-matched controls (Guilbert et al., 1989; Janowska-Wieczorek et al., 1991; Kacinski et al., 1989a,b, 1990). In patients with hematologic malignancies, remission is often reflected in a decreased or normalized serum CSF-1 concentration (Janowska-Wieczorek et al., 1991). In women

with ovarian carcinoma, the serum CSF-1 level is as predictive of tumor recurrence as is the serum CA-125 level. Employed together, these markers diagnose recurrence of ovarian carcinoma with an accuracy of 94% (Kacinski et al., 1990).

Immunoreactive CSF-1 and/or CSF-1 transcripts have been detected in carcinomas of the lung, breast, pancreas, endometrium, and ovary (Wu and Yunis, 1980; Kawasaki et al., 1985; Horiguchi et al., 1988; Kacinski et al., 1988, 1989a, 1991). Infiltrative breast lesions may also express c-fms transcripts or immunoreactive CSF-1R suggesting that CSF-1 may function as a paracrine or autocrine growth factor in breast cancer (Kacinski et al., 1991). In carcinomas of the breast and endometrium, expression of the CSF-1R may also herald a more aggressive phenotype (Kacinski et al., 1988, 1991; Baiocchi et al., 1991).

These data demonstrate that serum CSF-1 determinations may be clinically useful for the early detection and management of specific malignancies. Additionally, in tumors that produce CSF-1, coexpression of the CSF-1R may be of biological and prognostic significance.

CSF-1 AND HUMAN GLIOMA

An examination of CSF-1 responsive cells (i.e., trophoblast, macrophage, metastatic carcinoma) reveals a common characteristic—all infiltrate solid tissues. As astroglial expression of CSF-1 has been established, it seemed plausible that activation of the CSF-1R gene in transformed astrocytes might account for the infiltrative nature of glioblastoma. Initially, we employed a specific radioimmunoassay (Das et al., 1981) to quantify the CSF-1 content of culture medium conditioned by seven human glioblastoma cell lines. All seven cell lines, as well as a primary culture of encephalocele-derived, normal human astrocytes (Alterman et al., 1991), secreted significant quantities of CSF-1 into their culture medium; however, these levels did not correlate with in vitro tumorigenicity as determined by subcutaneous tumor formation in athymic mice (Alterman et al., in preparation). Northern blot analyses revealed that all of the cell lines as well as the primary astrocyte culture expressed the 4.5 kb CSF-1 mRNA, supporting the RIA findings. Total cellular RNA derived from intraoperative glioma samples also contained CSF-1 transcripts, indicating CSF-1 expression in cultured glia is not artifactual.

We next examined CSF-1 receptor expression in our glioma cell lines and intraoperative specimens. Northern blot analysis failed to reveal c-fms transcripts in any of our glioma cell lines. In addition, a radioreceptor assay failed to detect specific binding of ^{125}I-CSF-1 to our best characterized cell line, SNB-19 (Gross et al., 1988). Northern blot analysis did, however, reveal CSF-1 transcripts in operative specimens of human glioma, suggesting that CSF-1 may function as a paracrine or autocrine growth factor in some gliomas.

In order to determine if circulating CSF-1 levels could be employed as a marker for glioma, serum samples taken from patients with a variety of brain tumors were subjected to our radioimmunoassay. No patient with a glial or nonglial brain tumor exhibited elevated serum CSF-1 levels (Alterman et al., manuscript in preparation).

These data indicate that both normal and transformed human astrocytes produce CSF-1 in vivo and in vitro; however, the function of CSF-1 in normal and malignant neural tissue remains obscure. Most simply, one might argue that enhanced CSF-1 expression in malignant astrocytes reflects their derivation from a parent population that normally produces CSF-1. One might also hypothesize that enhanced CSF-1 production would be deterimental to tumor progression since CSF-1 functions as a chemoattractant and activator of macrophages. No data exist, however, to suggest that glioma CSF-1 levels correlate with mononuclear cell density within a tumor or with prognosis.

In order to prove that a malignant cell population is employing a factor as an autocrine stimulator of growth, one must demonstrate the following:

1. The cells express the growth factor;
2. The cells produce the intact, functional receptor; and
3. Perturbations of the growth factor/receptor system inhibit cellular proliferation.

Despite multiple claims of the identification of autocrine stimulators of glioma growth, these criteria have only been demonstrated for bFGF (Morrison et al., 1990).

We have fulfilled the first criterion, demonstrating CSF-1 production by human astrocytes and astrocytic tumors both in vitro and in vivo. We have also demonstrated CSF-1R gene expression in some human gliomas. The receptor's absence from glioma cell lines despite its detection in glioma tissue samples may be explained in a number of ways. The receptor may have been lost because of unknown selective pressures in vitro. Serum contains a variety of growth factors, known and unknown, that may stimulate the growth of specific subpopulations within a glioma. In this way, the CSF-1R-bearing subpopulation might be overgrown by others. Alternatively, one could hypothesize that CSF-1 activation occurs in a small subpopulation of glioma cells, those that infiltrate the brain parenchyma to create satellite lesions and distant metastases. Glioma cell lines are routinely derived from the primary lesion where CSF-1R-bearing cells may be scarce. Lastly, CSF-1R transcripts in primary tumor samples may be derived from tumor-infiltrating mononuclear cells, cells that are overgrown in vitro by the more proliferative malignant astrocytes.

No matter what the explanation, absence of the CSF-1R from our glioma cell lines presents an obstacle to the continued study of this growth factor in the pathogenesis of malignant glioma. In an attempt to overcome this obstacle, one could establish fresh glioma cultures in a

chemically defined medium containing CSF-1 as the sole mitogen. This medium would provide a selective growth advantage of CSF-1R-bearing cells and would improve the chances of establishing a permanent glioma cell line that expresses the receptor.

CONCLUSION

CSF-1 is a versatile factor both in form and function. Its presence in normal and malignant neural tissue is well established, although its function in the central nervous system is currently unclear. A more detailed examination of CSF-1 expression during morphogenesis, more extensive screening of CSF-1 and CSF-1R expression in human brain tumors, and the establishment of in vitro model systems are all required in order to properly study the function of this growth factor in normal neural development and in the pathogenesis of malignant glioma.

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