Role of the Colony-Stimulating Factor-1 Receptor (c-*fms*) and Its Ligand in Oncogenesis

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A. Introduction

An increasing body of experimental evidence supports the concept that malignant cell transformation results from mutations causing structural alteration or inappropriate expression of products that serve critical control functions in normal growth and development. Among the genes for growth regulatory molecules that might be targets for such genetic damage are those encoding polypeptide growth factors or their cell surface receptors. The macrophage colony-stimulating factor CSF-1 is a glycosylated polypeptide homodimer that stimulates the proliferation, differentiation, and survival of mononuclear phagocytes [1]. CSF-1 also appears to enhance the specialized immune effector functions of terminally differentiated monocytes and macrophages. The receptor for CSF-1 is the c-*fms* proto-oncogene product [2], an integral transmembrane glycoprotein with intrinsic protein-tyrosine kinase activity. The receptor is oriented in the plasma membrane with its ligand-binding domain exposed at the cell surface and its tyrosine kinase domain in the cytoplasm. Binding of CSF-1 activates the receptor kinase, which in turn initiates intracellular signals leading to the transcription of genes that effect the mitogenic response.

The current availability of the cloned genes for both CSF-1 and its receptor has facilitated studies on their function in normal hematopoiesis and oncogenesis. The transforming potential of structurally altered CSF-1 receptors has been established, and complementary DNAs encoding the receptor and ligand have been introduced into a variety of cell types. The long-term goal of these investigations is to define the normal function of the growth factor and its receptor and to determine whether alterations in their expression contribute to human malignancy. These studies provide the basis for a possible role of the CSF-1 receptor and its ligand in myeloid leukemogenesis.

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Structural Alterations Constitutively Activate the CSF-1 Receptor

The product of the feline retroviral oncogene v-*fms* retains the general domain structure of its c-*fms*-encoded progenitor: an aminoterminal ligand-binding portion linked by a single membrane-spanning segment to a carboxylterminal tyrosine kinase. However, differences in the amino acid sequence of the two molecules enable the v-*fms* gene product to function as a constitutive kinase and generate growth-promoting signals in the absence of the ligand. Although the v-*fms* product includes the complete extracellular domain and is able to bind CSF-1, its higher basal level of tyrosine kinase activity is only slightly increased by the ligand. Moreover, when introduced into murine hematopoietic cell lines that require specific growth factors for proliferation and survival in culture, the v-*fms* gene induced factor-independent growth and tumorigenicity by a nonautocrine mechanism [3, 4]. The ability to transform a CSF-1-dependent macrophage cell line and interleukin-3-dependent

myeloid cells did not result from autocrine production of the corresponding growth factors or transmodulation of their receptors by the v-*fms* gene product. Thus, the v-*fms* product is constitutively activated and can provide proliferative signals without binding the CSF-1 growth factor.

Introduction of v-*fms* into mouse bone marrow cells used to reconstitute lethally irradiated recipients resulted in proliferative disorders of multiple hematopoietic lineages [5]. The spleens of several primary recipients developed provirus-positive clones, which in some cases gave rise to clonal erythroleukemias or B-cell lymphomas when transplanted into secondary lethally irradiated hosts. Cells containing v-*fms* did not always have a proliferative advantage during serial transfers, suggesting that the development of these disorders was a multistep process in which expression of v-*fms* served as an initiating event. After a latency period, several other primary recipients developed myeloproliferative disorders which had clinical features reminiscent of chronic myelogenous leukemia but lacked evidence of clonality and were not efficiently transplanted to secondary hosts. Since diseases were observed in multiple hematopoietic lineages, the activity of this oncogene is not restricted to cells that ordinarily express CSF-1 receptors.

The v-*fms* gene efficiently transforms mouse NIH-3T3 fibroblasts to form colonies in semisolid medium and rendering them tumorigenic. Transduction of the human c-*fms* gene alone did not transform NIH-3T3 cells but did confer a CSF-1 responsive growth phenotype [6]. Cells expressing the normal CSF-1 receptor formed colonies in soft agar when plated in the presence of recombinant human CSF-1 and were morphologically transformed when cotransfected with a human CSF-1 cDNA. Thus, critical structural differences between the v-*fms* and c-*fms* gene products account for the transforming activity of the viral oncogene in the absence of the ligand.

The v-*fms* gene product differs from that of c-*fms* by several scattered amino acid changes and a carboxylterminal alteration that replaces 40 amino acids of the normal receptor with 11 unrelated residues in the oncogene product [7, 8]. The critical aspect of the C-terminal alteration appears to be the removal of a tyrosine residue at position 969, four amino acids from the receptor carboxylterminus. Chimeric v-*fms*/c-*fms* constructs demonstrated that this residue serves a negative regulatory function, possibly by limiting the activity of the receptor kinase in response to CSF-1. Substitution of the normal c-*fms*-encoded C-terminus for that of v-*fms* reduced the transforming efficiency of the oncogene more than tenfold [6, 9]. This inhibition was abrogated when the chimeric construct was prepared with a c-*fms* mutant encoding a phenylalanine residue in place of the wild-type tyrosine at position 969 [6]. Similarly, when NIH-3T3 fibroblasts were transformed by cotransfection of human c-*fms* and CSF-1 cDNAs, the efficiency of transformation was severalfold higher using a c-*fms* gene encoding the phenylalanine at position 969. However, the removal of tyrosine 969 by itself is insufficient to activate the transforming potential of the c-*fms* gene [6]. Rather, there appear to be one or more additional mutations in the v-*fms* gene that contribute to its oncogenic properties.

A chimeric construct, in which a major portion of the extracellular domain encoded by the v-*fms* gene was replaced by the corresponding region of the human c-*fms* gene, was markedly reduced in its transforming efficiency in the absence of CSF-1 [10]. However, like the normal c-*fms* gene product, the chimeric receptor efficiently induced transformation of NIH-3T3 cells when coexpressed with the ligand. Thus, despite the presence of the complete v-*fms*-coded kinase domain including its carboxylterminal truncation, the c-*fms*/v-*fms* chimera was to a large degree regulated by CSF-1. The simplest interpretation of these results is

that the exchanged portion of the extracellular domain contains one or more alterations critical to activation of the v-*fms* gene. Since the mutation(s) maps to the ligand-binding domain of the receptor, it may induce a conformational change that simulates the effect of binding CSF-1 and thus constitutively activates the receptor kinase. Site-directed mutagenesis in the c-*fms* gene should allow the identification of the specific alteration(s) in the extracellular domain that unmasks the latent transforming potential of the CSF-1 receptor.

These studies demonstrate that genetic alterations are able to activate the normal CSF-1 receptor to an oncogenic protein that transforms a variety of cells including those that do not ordinarily express this receptor. Efficient activation probably involves two events, one of which removes a negative regulatory tyrosine residue near the receptor carboxylterminus and a second that appears to mimic a ligand-induced conformational change.

C. Aberrant Expression of CSF-1 and Its Receptor

Colony-stimulating factor-1 was first shown to be synthesized by mesenchymal cells, including stromal cells of the bone marrow, and to interact with its receptor on mononuclear phagocytes [1]. Based on subsequent studies, the growth factor may also function in placental development during embryogenesis. CSF-1 is produced by uterine glandular epithelial cells in response to estrogens and progesterone during pregnancy [11], and its receptor appears to be expressed on placental trophoblasts [12]. The concentration of uterine CSF-1 increases during pregnancy with the highest levels being detected at term. It thus seems likely that the growth factor plays a role in the formation and maintenance of the placenta.

Coexpression of human CSF-1 and its receptor transforms immortalized NIH-3T3 fibroblasts by an autocrine mechanism [6]. Similarly, rearrangement and expression of the murine CSF-1 gene has been implicated as a secondary transforming event in a CSF-1-dependent murine macrophage cell line immortalized by the c-*myc* gene [13]. The autocrine transforming activity of CSF-1 and its receptor may be dependent on the type of cell in which they are expressed. For example, introduction of a human CSF-1 gene into a factor-dependent murine macrophage cell line resulted in CSF-1 independence but not tumorigenicity [14]. Since the v-*fms* oncogene fully transforms this same cell line, there must be critical differences between the normal CSF-1-mediated response and that induced by a constitutively activated receptor in the absence of ligand. It has recently been demonstrated that peripheral blood monocytes are induced to express CSF-1 in response to phorbol esters, inflammatory mediators such as γ-interferon and tumor necrosis factor, and other cytokines such as granulocyte/macrophage CSF [15, 16]. These results raise the possibility that regulated production of CSF-1 may stimulate the function of terminally differentiated mononuclear phagocytes by autocrine or paracrine mechanisms.

Expression of the CSF-1 receptor within the hematopoietic compartment is normally restricted to mononuclear phagocytes. Synthesis of this receptor is one of the earliest markers of monocytic differentiation, and low numbers of CSF-1 receptors are present on committed bone marrow progenitors. The receptor number increases about tenfold during normal monocytic differentiation, and high numbers (ca. 50000/cell) are maintained on mature monocytes and macrophages. In the presence of interleukin-3 or hemopoietin-1 (the macrophage product, IL-1α), even more primitive bone marrow precursors are rendered responsive to the growth factor [17, 18]. Aberrant expression of the CSF-1 receptor in cells that do not ordinarily express c-*fms* might enable them to be stimulated by the ligand and confer a proliferative advantage.

The murine c-*fms* locus has been identified as a preferred integration site for a retrovirus that causes myeloblastic leukemia [19]. Friend murine leukemia virus (F-MuLV) induces this disease in mice with a latency of 6–12 months. High levels of expression of the complete CSF-1 receptor coding sequence in more than 20% of these cases is a consequence of proviral integration upstream of the first coding exon in the c-*fms* gene. These cells might be unusually sensitive to the ligand due to the high levels of CSF-1 receptor synthesis. A proliferative advantage could develop into frank leukemia by the accumulation of additional genetic alterations. Loss of the germ-line c-*fms* allele in some of the cases raises the possibility that these secondary transforming events might include mutations in the receptor gene itself that activate its transforming potential. Whatever the mechanism, expression of the c-*fms* gene as a consequence of proviral insertion in the system identifies this as an important initiating event in the leukemogenic process.

Recent surveys of human acute myelogenous leukemic (AML) cells have provided evidence for expression of CSF-1 or its receptor in some of these cases. Screening of AML blasts by in situ hybridization for mRNA revealed CSF-1 or c-*fms* transcripts in about one-half of the cases and expression of both mRNAs in cells from several patients [20]. A survey of human AML blasts by flow cytometry with monoclonal antibodies to the CSF-1 receptor demonstrated that approximately 30% of the pediatric cases expressed the c-*fms* gene product (R.A. Ashmun et al., manuscript submitted). CSF-1 receptors on the leukemic cells underwent downmodulation in response to the growth factor or phorbol esters, suggesting that they were functionally unaltered. As might be expected by the normally restricted distribution of CSF-1 receptors to mononuclear phagocytes, the highest percentage of positive cases was among leukemic cells that showed evidence of monocytic differentiation. However, in both studies c-*fms* expression was also observed in some AML cases that were undifferentiated or exhibited granulocytic differentiation. As in the F-MuLV-induced murine myeloblastic leukemia, aberrant CSF-1 receptor expression in early myeloid cells might be associated with the development of disease in these patients.

D. Discussion

Future investigations into a role of CSF-1 and its receptor in leukemogenesis must include studies on the regulation of their expression at the transcriptional and post-transcriptional levels. The soluble growth factor is derived by proteolytic cleavage of membrane-bound precursors, some of which are stably expressed at the cell surface [21]. Factors that influence the cleavage of CSF-1 precursors may represent an additional mechanism for regulation of CSF-1 function. In the human AML blasts that expressed CSF-1 transcripts, detectable quantities of the growth factor were secreted only when the cells were incubated with phorbol esters [20].

The long arm of human chromosome 2 contains the genes for several hematopoietic growth factors and cell surface receptors including CSF-1 at 5q33.1 and c-*fms* at 5q33.3–33.4. Acquired interstitial deletions of 5q are associated with a characteristic form of refractory anemia ("$5q^-$ syndrome") and AML that develops after exposure to toxic chemicals. A detailed molecular analysis of this portion of chromosome 5 will be required to determine whether genetic lesions contributing a human cancer involve either the coding or regulatory sequences of genes in this region.

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