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Signal transduction by the macrophage-colony-stimulating factor receptor (CSF-1R)

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SUMMARY

The macrophage-specific colony-stimulating factor 1 (CSF-1 or M-CSF) is required throughout the G_1 phase of the cell cycle to regulate both immediate and delayed early responses necessary for cell proliferation. These are triggered by the binding of the growth factor to the colony-stimulating factor 1 receptor and the activation of its intrinsic tyrosine-specific protein kinase. Phosphorylation of the colony-stimulating factor 1 receptor on specific tyrosine residues enables it to bind directly to cytoplasmic effector proteins, which in turn relay receptor-induced signals through multiple-signal transduction pathways. The activity of p21*ras* as well as transcription factors of the *ets* gene family appears to be required for colony-stimulating factor 1 to induce the c-*myc* gene, and the latter response is essential to ensure cell proliferation. Genes within the *fos/jun* or activator protein 1 family are targeted via a parallel and independently regulated signal transduction pathway. The continuous requirement for colony-stimulating factor 1 after the immediate early response is initiated indicates that expression of additional delayed early response genes, although contingent on previously induced gene products, might also depend on colony-stimulating factor 1-induced signals. Among the growth factor-regulated delayed early response genes are D-type G_1 cyclins, which play an important role in cell-cycle progression.

Key words: CSF-1, CSF-1R, signal transduction

INTRODUCTION

Macrophage colony-stimulating factor (M-CSF or CSF-1) is a lineage-restricted growth factor that regulates proliferation, differentiation, and survival of monocytes, macrophages, and, their early bone-marrow progenitors (Sherr and Stanley, 1990). It is a homodimeric glycoprotein produced by fibroblasts and mesenchymal cells in biologically active secreted and membrane-bound forms (Rettenmier and Roussel, 1988). CSF-1-deficient mice (op/op) develop osteoporosis because they lack the ability to produce osteoclasts, which are macrophage-derived. Nevertheless, this defect can be corrected by CSF-1 administration (Wiktor-Jedrzejczak et al., 1990). CSF-1 is also involved in placental and fetal development (Pollard et al., 1987; Regenstreif and Rossant, 1989).

CSF-1 mediates its effects by binding to a single high-affinity transmembrane glycoprotein receptor (CSF-1R) with intrinsic tyrosine kinase activity (Roussel and Sherr, 1993). CSF-1R, the *fms* proto-oncogene (Sherr, 1988; Rohrschneider and Woolford, 1992) is a member of a growth factor receptor family that includes the platelet-derived growth factor receptors (PDGF-R, A and B), stem cell factor receptor (c-*kit*), and *flk2/flt3* proteins (Claesson-Welsh et al., 1989; Matsui et al., 1989; Qiu et al., 1988; Mathews et al., 1991). Each of these receptors is characterized by a unique kinase insert (KI) domain, which can serve as an anchor for several signaling proteins.

Growth factor binding and subsequent activation of the receptor trigger a series of events that ultimately leads to mitosis. Removal of growth factors results in cell cycle arrest, while prolonged starvation leads to cell death by apoptosis. However, restimulation by specific growth factors or serum following short periods (12-18 hours) of growth factor deprivation allows re-entry into the cell cycle and initiates the expression of a series of immediate early genes (including *fos*, *jun* and *myc*). Transcription of delayed early genes follows. These includes the G_1 D-type cyclins and their catalytic partners, which play an important role in G_1 progression and initiation of DNA synthesis (Sherr, 1993).

CSF-1R SIGNAL TRANSDUCTION

Ectopic expression of the human CSF-1R into naïve murine cell lines, including fibroblasts and IL-3-dependent myeloid cells, enables them to respond mitogenically to human CSF-1 and mimic macrophage signaling (Roussel and Sherr, 1989; Kato et al., 1989; Kato and Sherr, 1990). Transduction of the human CSF-1R gene into the murine fibroblast cell line NIH-3T3, rendered the cells dependent on human CSF-1 for growth and abrogated their requirement for PDGF, insulin growth factor (IGF), and epidermal growth factor (EGF) (Roussel and Sherr, 1989). Although fibroblasts secrete CSF-1, the murine growth factor binds with low affinity to the human CSF-1R

and is unable to activate the receptor's intrinsic tyrosine kinase, thereby precluding an autocrine loop. Hence, the transduced cells can divide indefinitely in chemically-defined medium with human CSF-1 as the sole growth factor present. This enables us to study the immediate and early responses triggered by the interaction of CSF-1 with its receptor in the absence of other stimuli. Further, these CSF-1R-transduced fibroblasts express an equivalent number of PDGF receptors, which can serve as a control in CSF-1 signal transduction studies.

On binding CSF-1, the receptor undergoes dimerization, activation of its intrinsic tyrosine kinase, and phosphorylation *in trans* of specific tyrosine residues: at amino acid 561, on the cytoplasmic side of the transmembrane domain (S. A. Courtneidge et al., unpublished results); at amino acids 699, 708 and 723, in the kinase insert domain, and at amino acid 809 in the core kinase domain (Clark et al., 1992; Shurtleff et al., 1990; Roussel et al., 1990). These phosphotyrosine residues then serve as 'magnets' for anchorage and activation of *src* homology-2 (SH2) motif-containing cytoplasmic effector proteins. The effectors, which may also be phosphorylated, are thought to transmit mitogenic signals. Several SH2-containing proteins bind to members of the CSF-1R family with diverse affinities (for review see Cantley et al., 1991; Koch et al., 1991; Songyang et al., 1993). They include: (1) phospholipase C-γl (PLCγl) (Anderson et al., 1990); (2) GTPase activating protein (GAP) (Reedijk et al., 1990); (3) *src* family proteins (*src, fyn* and *yes*)(Courtneidge et al., 1993); (4) the *sem5*/GRB2 gene product, a 25 kDa adaptor that constitutively binds and activates the *ras* guanine nucleotide exchange factor SOS-1 (Lowenstein et al., 1992; Clark et al., 1992; Bowtell et al., 1992); (5) SHC, another adaptor protein (Pelicci et al., 1992); and (6) the 85 kDa subunit of phosphotidylinositol 3 kinase (PI-K3) (Cantley et al., 1991).

Unlike the activated form of the PDGF-receptor, which associates with most of the above effectors, CSF-1R binds relatively few signal transducing proteins. It interacts with the *src* family of tyrosine kinases (Courtneidge et al., 1993), as well as PI-3K (Shurtleff et al., 1990) and GRB2 (Van der Geer and Hunter, 1990), the binding sites for which have been mapped to the KI domain at Tyr723 and 699, respectively (Van der Geer et al., 1990). However, binding and activation of *src* family kinases by CSF-1R requires phosphorylation of the receptor on both Tyr809 and on Tyr561 (Courtneidge and Roussel, unpublished results; Courtneidge et al., 1993). To date, no other SH2-containing cytoplasmic effector has been shown to interact directly with the activated CSF-1R. This would be analogous to the situation for the activated insulin receptor (Tobe et al., 1993).

Mutating specific tyrosine residues in CSF-1R selectively affected different pathways required for mitogenic signaling. Deletion of the receptor's entire kinase domain does not significantly affect v-*fms* transformation or CSF-1-induced cell proliferation suggesting that tyrosines 699, 708 and 723 (which occur in the PI3-K and GRB2 binding sites), are not essential for mitogenesis (Shurtleff et al., 1990). In contrast, substituting phenylalanine for tyrosine at amino acid 809 severely inhibited CSF-1R-induced cell growth. Cells expressing the mutant receptor were blocked early in G_1 (Roussel et al., 1994), failing to grow in chemically-defined medium that contains CSF-1 or to form colonies in agar, despite retaining wild-type kinase activity and PI3K binding capability (Roussel et al., 1990).

The role of *src* family kinases in CSF-1R signaling

The *src* family kinases, *src*, *fyn* and *yes*, are activated as a result of their association, via their SH2 domains, with phosphotyrosine residue 809 of CSF-1R (Courtneidge et al., 1993). Substitution of Tyr809 for phenylalanine prevents efficient binding and subsequent activation of *src*, suggesting that P-Tyr809 and its flanking sequences play an essential role in CSF-1 proliferation. Microinjection of an antibody (anti-*cst*-1) recognizing all three of the above *src* kinases, inhibited S phase entry as measured by BrdU incorporation into DNA. This inhibition was maximal when CSF-1R wild-type-expressing cells were injected between 0 and 6 hours after re-entry into the cell cycle. However, the antibody had no effect when injected after mid-G_1. This suggests that activation of the *src* kinase family is critical and essential for CSF-1 signaling early in G_1, but dispensable, several hours prior to S phase commitment (W. Roche, M. Koegl, M. F. Roussel and S. A. Courtneidge, unpublished results).

CSF-1R signaling and *ras*

CSF-1 induces an increase in guanine nucleotide triphosphate (GTP) bound to p21*ras* in cells expressing wild-type CSF-1R (Gibbs et al., 1990). Indeed, microinjection of anti-$p21^{ras}$ antibodies inhibits v-*src*, v-*fms* and v-*ras* transformation (Stacey et al., 1991). GAP, a downstream regulator of *ras* activity, inhibits CSF-1-mediated proliferation as demonstrated by overexpression of the full-length or merely the catalytic domain of *ras* GAP (Bortner et al., 1991). GAP neither binds to CSF-1R nor undergoes tyrosine phosphorylation in proliferating macrophages (Reedijk et al., 1990), suggesting that signaling through p21*ras* might be mediated by an unknown adaptor, or by GRB2, binding to the activated receptor. However, GRB2 was found to bind to Tyr699, which we showed was not required for mitogenic signaling in NIH-3T3 fibroblasts (Van der Geer and Hunter, 1990; Shurtleff et al., 1990). Indeed, in cells harboring the CSF-1R(Phe809) mutant, we were unable to detect an active form of *ras* (J. Nathan Davis and M. F. Roussel, unpublished results), which suggests that CSF-1R activation of *ras* requires phosphorylation of Tyr809 and, potentially, other unmapped tyrosine-phosphorylated residues, and/or the binding of as yet uncharacterized signaling effector proteins.

The immediate early gene response

A single point mutation in CSF-1R substituting phenylalanine for tyrosine at codon 809 inhibits the mitogenic response to CSF-1 (Roussel et al., 1990). Transcription of the immediate early-response genes, c-*fos* and *jun*B, are induced, whereas c-*myc* expression is considerably reduced. However, ectopic expression of c-*myc* restored CSF-1-dependent proliferation indicating that c-*myc* function appears essential for CSF-1-dependent cell growth (Roussel et al., 1991). Further, these data suggest that mitogenic signaling by CSF-1R is mediated minimally by two independent pathways: one leading to c-*fos* and *jun*B transcription, and the other leading to c-*myc* transcription. The importance of these two signaling pathways in mitogenesis, particularly the c-*myc* pathway, has been demonstrated for other growth factor receptors, including the IL-2 receptor β chain (IL-2Rβ) (Shibuya et al., 1992), and the epidermal growth factor receptor (EGF-R) in the myeloid cell

line, BAF-BO3 (Shibuya et al., 1992), as well as for the *bcr-abl* oncogene (Sawyers et al., 1992). NIH-3T3 cells expressing CSF-1R (Phe809) can, therefore, be used as a genetic trap to identify cellular signaling molecules specifically involved in the *myc* pathway or which can bypass the requirement for *myc* transcription.

The activity of p21*ras* is required for the CSF-1R proliferative response by stimulating transcription from promoter elements containing binding sites recognized by the *ETS* family of transcription factors (Reddy et al., 1992). Enforced expression in CSF-1R-containing NIH-3T3 cells of the DNA-binding domain (DBD) of a human *ETS*-2 gene lacking a transactivation domain suppresses their CSF-1 responsiveness but does not affect the expression of c-*fos* and c-*jun*, immediate early genes. However, cells bearing CSF-1R (Phe809), have impaired c-*myc* expression. Ectopic expression of the c-*myc* gene overrides this suppressive effect and resensitizes the cells to CSF-1 (Langer et al., 1992). Conversely, cells expressing the CSF-1R (Phe809) can be complemented by the enforced expression of several *ETS* family members, including *ETS*-1, and *ETS*-2 (Roussel et al., 1994; Langer et al., 1992), *fli*-1 and EWS-*fli*-1 (M. F. Roussel and J. Ghysdael, unpublished results), as well as *elf*-1 (J. N. Davis and M. F. Roussel, unpublished results). However, these cells grow more slowly in CSF-1 than those expressing wild-type CSF-1R, suggesting either that: (1) *ETS* transcription factors must signal via the *myc* pathway to be fully active; or (2) other members of the *ETS* family are involved in the regulation of CSF-1-induced *myc* expression. Indeed, *ETS*-1 and *ETS*-2 transactivate reporter genes driven by the human and mouse c-*myc* promoters through a binding site that overlaps an E2F-1 site, required for E1A and serum-induced c-*myc* expression (Roussel et al., 1994). Although E2F-1 and *ETS* proteins share structural similarities in their DNA-binding domain and interact with similar consensus DNA-binding sites, E2F-1 and *ETS*-1 do not form heterodimers in vitro and do not transactivate c-*myc* synergistically (Roussel et al., 1994). These data suggest that E2F-1 and *ETS* factors may independently regulate c-*myc* through the same binding sites but at different times following growth factor stimulation.

Deregulation of the c-*myc* proto-oncogene is a hallmark of Ewing sarcoma (ES), a childhood bone and soft tissue tumor associated with a reciprocal translocation t(11;22)(q24;q12), which results in a fusion mRNA formed from the 5′ end of the gene on derivative (22) (EWS) and the 3′ DNA-binding portion of the *fli*-1, gene from chromosome 11. A member of the *ETS* family, *fli*-1 is rearranged and overexpressed in 75% of erythroleukemias induced in newborn mice infected by the replication-competent Friend leukemia virus (Ben-David et al., 1991). In line with the elevated expression of c-*myc*, EWS-*fli*-1 has been found to upregulate the activity of a human c-*myc* promoter/reporter construct (Bailly et al., 1994).

FUTURE PERSPECTIVES

Cells expressing the CSF-1R mutants offer a unique system to dissect different signal transduction pathways that mediate mitogenicity in response to CSF-1. Appropriately engineered cell lines should enable us to identify the cellular effectors that relay signals through these specific pathways.

Because cells expressing the CSF-1R (Phe809) can survive in the presence of CSF-1 without proliferating, they can be used as a genetic trap to identify regulators that govern cell cycle entry and progression in response to CSF-1. Complementation of the signaling-defective CSF-1R (Phe809) has already proven to be an effective means of identifying genes in the *myc* pathway. This strategy could also pinpoint genes downstream of *myc* that are essential for CSF-1R-induced mitogenesis such as the D-type cyclins.

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