Signal Transduction Through Foreign Growth Factor Receptors and Oncogenes Transfected into Interleukin-3-Dependent Hematopoietic Cells

J. H. Pierce [1]

Signal transduction mediated by growth factor interaction with specific membrane receptors is of critical importance in the regulation of normal cell growth and differentiation. Moreover, increasing evidence indicates that aberrations in these pathways are important in the neoplastic process. Factor-dependent hematopoietic cell lines provide a potentially important means of dissecting differences in growth factor regulatory pathways. In many cases, these lines can either proliferate or differentiate in response to several different hematopoietic cytokines. One such line, 32D, is strictly dependent on interleukin-3 (IL-3) for growth, possesses a normal diploid karyotype, displays an immature myeloid phenotype, and is nontumorigenic in nude mice. Granulocytic colony-stimulating factor (G-CSF) induces terminal neutrophilic differentiation of 32D cells when IL-3 is withdrawn from the culture. 32D cells do not express receptors for macrophage-CSF (CSF-1). Moreover, these cells are devoid of growth factor receptors which regulate cells of connective tissue origin, such as epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) receptors.

The molecular cloning of tyrosine kinase-containing growth factor receptor genes has made it feasible to investigate the ability of these specific receptors to couple with intracellular components needed to evoke a functional response in hematopoietic cells which do not normally express these growth factor receptors. We have introduced expression vectors for several tyrosine kinase-containing growth factor receptors and oncogenes into the 32D cell line to investigate signaling pathways through which they may couple.

An expression vector for the EGF receptor (EGFR) was introduced into the 32D myeloid cell line which is devoid of EGFRs and absolutely dependent on IL-3 for growth [1]. Expression of the EGFR allowed these cells to utilize EGF for transduction of a mitogenic signal (Table 1). When the transfected cells were propagated in EGF, they exhibited a more mature myeloid phenotype than was observed under conditions of IL-3-directed growth. Moreover, exposure to EGF led to a rapid stimulation of phosphoinositide (PI) metabolism, while IL-3 had no detectable effect on PI turnover. Although the transfected cells exhibited high levels of functional EGFRs, they remained nontumorigenic. In contrast, transfection of v-*erb*-B, an amino-terminal-truncated form of the EGFR, not only abrogated the IL-3 growth factor requirement of 32D cells (Table 1), but caused them to become tumorigenic in nude mice. These results showed that a naive hematopoietic cell expresses all of the intracellular components of the EGF-signaling pathway necessary to evoke a mitogenic response and sustain continuous proliferation.

Distinct genes encode α and β PDGF receptors that differ in their abilities to be triggered by three dimeric forms of the

[1] Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, Maryland 20892, USA.

Haematology and Blood Transfusion Vol. 35
Modern Trends in Human Leukemia IX
R. Neth et al. (Eds.)

Table 1. Mitogenic response and cloning efficiency of 32D transfectants

Cell line	Treatment	SI of ^{3}[H]TdR incorporation	(%) Cloning efficiency
32D	IL-3 + serum	234.5	35.5
	EGF + serum	1.0	< 0.1
	Serum	1.0	< 0.1
32D-v-*erb* B	IL-3 + serum	225.5	28.0
	EGF + serum	231.0	30.5
	Serum	228.0	28.5
32D-EGFR	IL-3 + serum	215.5	27.5
	EGF + serum	185.5	25.0
	Serum	1.0	< 0.1

The cell proliferation assay was performed as described [1]. Results are expressed as a stimulation index. Data are the mean of duplicate samples. The cloning efficiency was established by plating cells at various concentrations in growth medium supplemented with 15% FCS and 0.48% sea plaque agarose. IL-3 (50 U/ml) or EGF (100 mg/ml) was included when specified. Visible colonies were scored at 12 days after plating.

SI: stimulation index; *cpm ^{3}[H] TdR* incorporation with growth factor and/or serum/*cpm ^{3}[H]* incorporation with serum.
FCS: fetal calf serum.

PDGF molecule. We show that PDGF receptor mitogenic function can be reconstituted in IL-3-dependent 32D cells by introduction of expression vectors for either α or β PDGF receptor cDNAs into this naive hematopoietic cell line [2]. Thus, each receptor is independently capable of coupling with mitogenic signal transduction pathways inherently present in these cells. Activation of either receptor also resulted in chemotaxis, alterations in inositol lipid metabolism, and mobilization of intracellular Ca^{2+}. The magnitude of these functional responses correlated well with the binding properties of different PDGF isoforms to each receptor. Thus, availability of specific PDGF isoforms and relative expression of each PDGF receptor gene product are major determinants of the spectrum of known PDGF responses.

The c-*fms* proto-oncogene encodes the receptor for CSF-1. Expression vectors containing either normal or oncogenic point-mutated human c-*fms* genes were transfected into IL-3-dependent 32D cells in order to determine the effects of CSF-1 signaling in this murine clonal myeloid progenitor cell line [3]. CSF-1 was shown to trigger proliferation in association with monocyte differentiation of the 32D-c-*fms* cells. Monocytic differentiation was reversible upon removal of CSF-1, implying that CSF-1 was required for maintenance of the monocyte phenotype but was not sufficient to induce irrevocable commitment to differentiation. Human CSF-1 was also shown to be a potent chemoattractant for 32D-c-*fms* cells, suggesting that CSF-1 may serve to recruit monocytes from the circulation to tissue sites of inflammation or injury. Although c-*fms* did not release 32D cells from factor dependence, point-mutated c-*fms* [S301, F969] was able to abrogate their IL-3 requirement and induce tumorigenicity. IL-3-independent 32D-c-*fms* [S301, F969] cells also displayed a mature monocyte phenotype, implying that differentiation did not interfere with progression of these cells to the malignant state. All of these findings demonstrate that a single growth factor receptor can specifically couple with multiple intracellular signaling pathways and play a critical role in modulating cell proliferation, differentiation, and cell migration.

Proliferation and maturation of hematopoietic cells is a complex but orderly process involving growth factor-controlled programs that lead to self-renewal or terminal differentiation. Although many of the genes encoding growth factors and several of their receptors have been cloned, the intracellular mechanisms of action of these signaling pathways are not well understood. The recent cloning of IL-3 and G-CSF receptors has revealed that these genes do not contain tyrosine kinase-like domains. However, it has been demonstrated that at least IL-3 does stimulate the tyrosine phosphorylation of specific intracellular substrates in murine cell lines.

The fact that 32D is a clonal myeloid precursor cell line has raised the question as to whether signals transduced by all activated receptor tyrosine kinases might interact with the same specific intracellular substrates. After introduction of the tyrosine kinase receptors into 32D cells, they showed evidence of proliferation as well as partial myeloid maturation in response to their respective ligands. Since IL-3 only induces proliferation in this system and G-CSF strictly triggers terminal differentiation of these cells to mature neutrophilic granulocytes, it may now be possible to correlate specific alterations in second messenger systems or tyrosine-phosphorylated substrates involved in regulating specific proliferation and/or differentiation pathways within the myeloid lineage.

References

1. Pierce JH, Ruggerio M, Fleming TP, DiFiore PP, Greenberger JS, Varticouski L, Schlessinger J, Rovera G, Aaronson SA (1988) "Signal transduction through the EGF receptor transfected into interleukin-3-dependent hematopoietic cells." Science 239:628–631
2. Matsui T, Pierce JH, Fleming TP, Greenberger JS, LaRochelle WJ, Ruggerio M, Aaronson SA (1989) "Independent expression of human α or β platelet-derived growth factor receptor CONAs in a naive hematopoietic cell leads to functional coupling with mitogenic and chemotactic signaling pathways." Proc Natl Acad Sci USA 86:8314–8318
3. Pierce JH, Marco E, Cox GW, Lombardi D, Ruggerio M, Varesio L, Wang LM, Chondhury GG, Sakaguchi AY, DiFiore PP, Aaronson SA (1990) Macrophage-colony-stimulating factor (CSF-1) induces proliferation, chemotoxis and reversible monocytic differentiation in myeloid progenitor cells transfected with the human c-*fms*/CSF-1 receptor CDNA 1990. Proc Natl Acad Sci USA 81:5613–5617