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REVIEW

DYSREGULATION OF GROWTH FACTOR-RECEPTOR SYSTEM IN CELLULAR TRANSFORMATION

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INTRODUCTION

In the past few years, molecular biological and biochemical studies have led to a tremendous accumulation of information concerning the control mechanisms of cell growth and differentiation. In conjunction with these studies, evidence has been accumulated which supports the idea that dysregulation of the growth factor-receptor system is deeply involved in the malignant transformation of the cells. In fact, several oncogene products turned out to be identical or analogous to cellular proteins functioning as growth factors or their receptors. Many of these findings also support the concept of autocrine growth, which predicts that constitutive production and consumption of growth factor in a given cell is one of the general mechanisms for the acquisition of cell growth autonomy and transformation.[1] More recently, with the advent of recombinant DNA technology, it has become possible to examine directly whether dysregulation of the growth factor-receptor system leads to the acquisition of growth autonomy at the molecular level by introducing growth factor and/or the receptor genes into appropriate cells.

In this review, we shall give an overview of the growth factors and their receptors in the context of their oncogenic properties and discuss the possible role of these molecules in the development of malignant cells.

INVOLVEMENT OF GROWTH FACTORS AND RECEPTORS IN CELLULAR TRANSFORMATION

1. Platelet-derived Growth Factor

Evidence that growth factor is directly involved in malignant transformation of cells was first obtained in the case of platelet-derived growth factor (PDGF). PDGF, a 30 kd protein composed of two different peptide chains denoted A and B, stimulates the proliferation of mesenchymal cells.[2–4] The two chains are disulfide-linked and the dimerization is essential for biological activity. The major sources of PDGF *in vivo* are platelets and macrophages.

Amino acid sequence analysis of PDGF revealed that the B-chain is a cellular counterpart of the transforming protein ($p28^{v\text{-}sis}$) encoded by v-*sis*, an oncogene of simian sarcoma virus (SSV).[5–9] The SSV induces various tumors including fibrosarcomas and glioblastomas, both of which express the PDGF receptor (PDGF-R). The transforming phenotype is effectively blocked by anti-PDGF.[10, 11] Almost identical results were obtained through transfection and expression of cDNA encoding normal PDGF-B chain.[12, 13] These results unequivocally show that normal growth factor manifests transforming activity when aberrantly expressed in a certain type of cells.

It is well known that naturally occurring cancer cells produce factors having PDGF-like activity.[4] One of them, which is produced by a human osteosarcoma line, has been identified as a homodimer of the PDGF-A chain. The growth of the osteosarcoma cell lines is dependent on the autocrine stimulation mediated by the PDGF-A homodimer molecules.[14, 15]

PDGF-R is a 180 kd transmembrane glycoprotein consisting of 1098 amino acids with an intracellular tyrosine kinase domain.[16] At present, there is no report that PDGF-R itself manifests oncogenic properties.

2. Epidermal Growth Factor/Receptor and Transforming Growth Factor-α

Epidermal growth factor (EGF) is a growth factor first described in 1962 by Cohen.[17] EGF binds to its specific cell membrane receptor (EGF-R) and delivers a mitogenic signal in fibroblasts. This molecule is a peptide consisting of 53 amino acids that is derived from a large amino acid precursor consisting of 1217 amino acids.[18, 19] The precursor is a transmembrane molecule, and matured EGF is believed to be cleaved from the precursor through as-yet unknown mechanism(s). Transforming growth factor-α (TGF-α) was originally identified in the culture medium of several fibroblast lines transformed by Moloney or Kirsten murine sarcoma virus.[20] The molecule has an ability to confer a transformed phenotype to non-transformed fibroblasts. This discovery has led Sporn and Todaro to propose a role of autocrine growth stimulation in cellular transformation.[1] TGF-α is a 50-amino-acid peptide derived from a membrane-associated 160-amino-acid precursor.[21, 22] It binds to the same receptor as EGF.[23, 24] Although this factor is produced by various transformed cell lines, it is also produced and utilized during embryogenesis.[25] The EGF-R is a membrane protein consisting of 1186 amino acids.[26] The molecule contains a single membrane-spanning region and a cytoplasmic domain with tyrosine kinase activity.

A close relation of the EGF/EGF-R system to cellular transformation became evident with the discovery that v-*erbB* oncogene encodes a truncated version of EGF-R.[27, 28] The v-*erbB* is an oncogene derived from avian erythroblastosis virus that induces erythroleukemias and sarcomas in chicken. The oncogene product, gp74$^{v\text{-}erbB}$, lacks most of the extracellular EGF binding domain and 32 amino acid residues at the carboxy terminal end of the receptor molecule. Such a truncation leads to dysregulation, i.e., constitutive activation of the tyrosine kinase, whose activity is otherwise controlled by the ligand-receptor interaction.[29] The dysregulated activation of the kinase domain may be responsible for the cellular transformation. The discovery also gave rise to an interesting and important question, i.e., whether or not the normal EGF-R itself has an oncogenic potential *per se* under certain circumstances. In this regard, involvement of EGF-R in the malignant process has been suggested from the observation that the receptor gene is amplified and overexpressed in a wide variety of human tumor cells (especially squamous cell carcinomas and glioblastomas) without overt structural alterations.[30–33] In fact, experiments with EGF-R-cDNA transfected NIH/3T3 have shown that overexpression of a normal EGF-R results in cellular transformation when EGF is supplied exogenously.[34, 35] Interestingly, such transformed NIH/3T3 cells are tumorigenic in nude mouse even in the absence of exogenous EGF.[34, 35]

EGF-R-related gene, designated c-*erbB-2/HER-2/neu*, was recently identified.[36–38] The gene encodes an EGF-R like transmembrane molecule with tyrosine kinase activity. The molecule has been speculated to function as a receptor for a heretofore unidentified growth factor. Amplification and overexpression of the c-*erbB-2* gene have been reported in adenocarcinomas.[39–41] Such overexpression of the gene product seems sufficient for NIH/3T3 transformation.[42] The ligand-independent transformation by c-*erbB-2* suggests that the *erbB-2* protein has an ability to deliver a certain level of mitogenic signals even in the absence of ligand. In the case of *neu*, a rat counterpart of human *erbB-2*, a single amino acid alteration within the transmembrane region has been shown to potentiate the transforming activity of this molecule.[43]

3. Bombesin/Gastrin-releasing Peptide and Bombesin-related Substances

Involvement of humoral growth factor in

the development of a certain type of lung cancer has been reported. Cell lines derived from small cell lung cancer produce and respond to bombesin-like growth factor in an autocrine manner.[44] Bombesin/gastrin-releasing peptide (GRP) has an ability to stimulate the growth of fibroblasts[45] as well as bronchial epithelial cells *in vitro*.[46] It has been demonstrated that a monoclonal antibody that binds to the carboxyl-terminal portion of bombesin and bombesin-like growth factor blocks the ligand/receptor interaction and leads to the inhibition of the cell growth both *in vitro* and *in vivo*.[44] This study not only clarified the role of growth factor in a certain type of lung cancer, but also raised the possibility that bombesin antagonists may have a therapeutic role in small cell lung cancer.

4. Fibroblast Growth Factors

Recent reports have demonstrated that molecules related to fibroblast growth factors (FGFs) have an oncogenic activity. FGFs are considered to have an important role in angiogenesis. So far, two related FGFs, basic and acidic FGFs, have been identified.[47] Recently, a novel oncogene designated *hst*[48] or *KS*[49] has been isolated independently by transfecting DNA from a human stomach cancer or Kaposi's sarcoma, respectively. The sequence analysis of *hst/KS* gene revealed that the gene product is a potent secretory protein having significant structural homology to FGFs. Since the novel oncogene could be activated by DNA rearrangement or truncation during the transfection and selection, the role of the oncogene in the original tumor development remains unclear at present. However recent genomic analysis revealed that acquisition of the oncogenic activity of *hst/KS* does not require any mutational events.[50] The fact that *hst* is one of the most frequently identified oncogenes in NIH/3T3 assay[51] implies that it is involved in a wide range of cell malignancies, that include gastro-intestinal cancers.

The oncogenic potential of FGF has also been documented. Basic FGF is a membrane-associated molecule and is not secreted. The cDNA analysis of the basic FGF revealed that the molecule lacks a signal sequence for secretion.[52] In order to examine whether or not overexpression of FGF results in cellular transformation, an expression plasmid which encodes a secretory form of basic FGF by connecting an immunoglobulin-derived signal sequence was constructed.[53] Transfection of the expression vector into NIH/3T3 resulted in the transformation of cells and the transformed phenotype is totally dependent on the autocrine stimulation by the secreted basic FGF.

A recently identified oncogene, *int-2*,[54] which is involved in mouse mammary tumor virus (MMTV) - mediated tumor development, is also a member of the FGF family.

5. Transforming Growth Factor-β (TGF-β)

Transforming growth factor-β (TGF-β) was originally identified as a humoral factor to stimulate several fibroblast cell lines in soft agar.[55] However, subsequent studies led to the conclusion that the factor should be re-evaluated as a negative growth factor, since this molecule often exhibits a strong inhibitory effect on growth in a wide range of cells.[56, 57]

Based on the observation that TGF-β acts as a powerful growth inhibitor, Sporn and Roberts extended the autocrine hypothesis by including the idea that malignant cellular transformation can occur when a cell fails to produce or respond to a specific negative growth factor(s).[58] In fact, several experimental systems including human breast cancer[59] and keratinocyte transformation[60] support this idea. It should be noted that other growth-inhibitory factors such as tumor necrosis factors (TNFs) and interferons (IFNs) may also have such a regulatory role in preventing abnormal proliferation of cells.

6. Colony-stimulating Factors

Colony-stimulating factors (CSFs) were initially identified on the basis of ability to promote the growth and differentiation of mouse myeloid progenitor cells in soft agar.[61, 62] So far, four different CSFs have been isolated and characterized in both mouse and man. Granulocyte-macrophage-CSF (GM-CSF) acts on granulocytes, eosinophils and macrophages, while macrophage-CSF (M-CSF, also called CSF-1) promotes proliferation, differentiation and activation of monocyte-macrophage lineage cells. Granulocyte-CSF (G-CSF) acts on granulocytes, and multi-

CSF, also called interleukin-3, functions not only on uncommitted bone-marrow stem cells but also on myeloid cells, erythroid cells and megakaryocytes.

a. GM-CSF

GM-CSF, a 14–35 kd glycoprotein produced by activated T cells, fibroblasts and endothelial cells, stimulates the proliferation of granulocytes, macrophages and eosinophils by binding with a specific membrane receptor.

The involvement of GM-CSF in the development of cell malignancy has been suggested by the observation that the growth of freshly isolated myeloid leukemic cells depends on exogenously added GM-CSF.[61] This observation raised the possibility that GM-CSF is involved in myeloid-monocytic leukemogenesis. In fact, introduction and expression of the human GM-CSF gene in a mouse GM-CSF-dependent cell line gave rise to the generation of factor-independent transformants which developed tumors in syngeneic mice.[63] Although cells used for this transfection experiment had been immortalized *in vitro* and may have received some genetic alterations contributing to the acquisition of malignant phenotype, the result demonstrates that continuous autocrine growth stimulation by this cytokine plays a role in the development of hematopoietic malignancy. An interesting observation in this experiment was that anti-GM-CSF failed to inhibit the growth of the transformed cells producing GM-CSF. Although the possibility exists that the transformed cells had lost their factor-dependency during *in vitro* and *in vivo* selection, one may speculate alternatively that the growth factor-receptor interaction occurred in intracellular compartments. If this is the case, such an "intracellular autocrine loop" may have some advantages over an "extracellular autocrine loop."

In contrast to the above experiment, a study with transgenic mouse carrying human GM-CSF gene provided another interesting insight into the role of GM-CSF in cellular transformation.[64] Though the transgene was actively transcribed and the serum GM-CSF level became high enough to induce eye, peritoneal and muscular lesions caused by GM-CSF-activated macrophages, those mice did not develop myeloid or monocytic leukemia.

b. CSF-1 and its receptor

CSF-1 (M-CSF) is produced by mesenchymal cells such as fibroblasts, endothelial cells as well as macrophages, and it is responsible for the growth, differentiation and activation of the mononuclear phagocytic cells. Recent studies with cloned cDNAs revealed that there are at least two forms of CSF-1; one is a 70–90 kd glycoprotein composed of a homodimer of 35–45 kd subunits and the other is a 40–50 kd homodimer assembled through interchain disulfide bonds.[65]

The v-*fms* oncogene of the Susan McDonough strain of feline sarcoma virus has an ability to transform fibroblasts, macrophages and myeloid cells.[66] The normal cellular counterpart of the v-*fms* gene was molecularly cloned and shown to encode a transmembrane glycoprotein, $gp170^{c\text{-}fms}$, having a tyrosine kinase domain.[67] The overall structure of c-*fms* shows striking homology with PDGF-R[16] and v-*kit* oncogene of HZ4 feline retrovirus.[68] Sherr *et al.* reached the conclusion that the c-*fms* product is the CSF-1 receptor that is expressed on the surface of monocyte-macrophages.[69] In contrast to the cases of EGF-R and v-*erbB*, both v- and c-*fms* (CSF-1 receptor) can bind the ligand with high affinity. While the tyrosine kinase of c-*fms* gene product needs to be activated by the extracellular signaling of CSF-1, the corresponding kinase activity of the v-*fms* product is constitutive. The NIH/3T3 assay revealed that transformation of cells with c-*fms* required exogenous CSF-1,[70] while transformation with v-*fms* did not, suggesting again a relation between the acquisition of malignant phenotype and constitutive activation of the receptor-associated tyrosine kinase.

Although the physiological distribution of CSF-1 receptor is restricted to cells of macrophage lineage, a recent bone marrow rescue experiment with hematopoietic stem cells infected *in vitro* by retrovirus carrying expressible v-*fms* demonstrated that the v-*fms* gene product potentially has an ability to transform various hematopoietic cells including B-lymphocytes (B cells), myeloid cells and macrophages.[71]

7. Interleukins

a. Interleukin-1

Interleukin-1 (IL-1), originally defined as a

monocyte-derived factor mitogenic for thymocytes, has many biological activities upon various cells. IL-1 induces fever and acts as an important inflammatory mediator by releasing prostaglandin E_2 and inducing the synthesis of acute-phase proteins. Molecular cloning of IL-1 cDNA revealed the existence of two different molecules, IL-1α and IL-1β, with similar molecular weights of 21 kd.[72,73] Both IL-1α and β molecules bind to an identical cellular receptor of 80 kd.[74–76] IL-1 has been shown to support the growth of fibroblasts, astroglial cells as well as mesangial cells, but inhibits the growth of certain tumor cells such as melanoma.[77,78] There are several reports indicating that IL-1 is involved in the growth of brain tumors, especially astrocytoma, based on the observation that an astrocytoma line produces and responds to IL-1.[79]

b. Interleukin-2

Interleukin-2 (IL-2) is a humoral factor produced and secreted by activated CD4-positive (helper) T lymphocytes (T cells).[80–82] This factor is essential for long-term maintenance of non-transformed T cells *in vitro*. Matured human IL-2 is a 15 kd glycoprotein with 133 amino acids having a single intramolecular disulfide bridge that is essential for biological activity.[83] The IL-2 gene consists of 4 exons[84] and is located on chromosome 4q in man.[85] An activated T cell antigen with a molecular weight of 55 kd (p55), previously referred to as Tac antigen,[86] was shown to be the IL-2 receptor (IL-2R); it consists of 251 amino acids.[87–90]

The IL-2/IL-2R system is unique among various growth factor-receptor systems in that both ligand and receptor are transiently induced following antigenic stimulation of T cells.[81,82] Thus, in effect, clonal proliferation of antigen-stimulated T cells by the IL-2/IL-2R system is guaranteed. Recent studies have clarified DNA sequences required for T cell-specific activation of IL-2 and IL-2R (p55) genes upon stimulation with mitogen. These sequences are located 5′-upstream of the first exon of the genes, but do not have significant sequence homology to each other.[91–93]

Activated T cells manifest high- and low-affinity IL-2Rs, both of which contain the Tac antigen (hereafter termed p55 for convenience).[94] The IL-2 signal seems to be transduced only through the high-affinity IL-2R. Although the p55 molecule has an IL-2 binding activity, the molecule itself has low-affinity for IL-2.[88–90] In addition, the cytoplasmic domain of p55 consists of only 13 amino acids. Hence, at present, the mechanism that determines receptor affinity and transduces the IL-2 signal inside the cell is not fully understood. Recent studies indicate that high-affinity IL-2 receptor requires molecules other than p55 to form the functional receptor complex.[95–99] A newly identified IL-2 binding molecule called p75, which is mainly expressed on NK lineage cells, is a possible candidate as a component of the high-affinity IL-2 receptor complex.[100–102]

Involvement of the IL-2/IL-2R system in T cell malignancy, especially in relation to adult T cell leukemia (ATL), has been well documented in the past several years. ATL is a fatal hematological disorder arising from a monoclonal proliferation of matured CD4-positive (i.e. helper) T cells[103] that is endemic in south-west Japan and the Caribbean islands. This disease is particularly significant in that a retrovirus, human T-cell leukemia virus type-1 (HTLV-1), was identified for the first time as the causative agent for human cancer.[104] While HTLV-1 does not contain a typical oncogene in its proviral genome,[105] it can rapidly transform T cells *in vitro*.[106] The relationship of the IL-2 system with the development of ATL has been suggested by the observation that, in almost all cases, ATL cells express relatively large amounts of IL-2R (p55) molecules on the cell surface[103] and some HTLV-1-transformed T cell lines produced and responded to IL-2.[107] The observation initially led to a hypothesis that ATL leukemogenesis involves the IL-2 autocrine mechanism.[107] Subsequent studies, however, revealed that most T cells transformed *in vitro* with HTLV-1 did not produce a detectable level of IL-2.[108]

The role of viral proteins in the T cell transformation by HTLV-1 has been studied and a protein designated *tax-1* (*tat-1*, $p40^x$) has been demonstrated to function as a *trans*-acting transcriptional activator for the virus LTR.[109–112] Subsequent studies revealed that the *tax-1* also activates the cellular gene encoding IL-2R (p55) by indirectly affecting the DNA sequence required for IL-2R (p55) gene

activation.[92, 113] This observation offered a molecular basis for the constitutive expression of IL-2R (p55) on ATL cells as well as HTLV-1-transformed T cell lines. In addition, *tax-1* has been shown to activate the IL-2 gene, and the activation seems to be synergistically potentiated by delivering a mitogenic signal through T cell receptor-T3 complex triggering.[92] In fact, the possibility that ATL cells are constitutively activated *in vivo* by a still-unknown antigen(s) has been suggested from the observation that T cell receptor-T3 complex is down-modulated from the ATL cell surface.[114] Accordingly, in contrast to the results obtained from established ATL cells and HTLV-1 transformed cell lines, those data derived from *tax-1* studies indicate that aberrant activation of IL-2 and IL-2R genes by *tax-1* may allow the HTLV-1-infected helper T cells to grow by an autocrine mechanism. In view of the above results, such an autocrine stimulation may be further enhanced when a given virus-infected T cell clone is triggered by antigen (due to the synergistic activation of the IL-2 gene), thereby bringing the cell to a predisposed state for the acquisition of further malignant characteristics. In addition, some leukemic cells freshly isolated from acute, but not chronic, ATL patients produce and respond to IL-2 in an autocrine fashion.[115] This may indicate that the IL-2/IL-2R system is also involved in the acute acceleration (crisis) of ATL. In this context, it has been recently demonstrated that an IL-2 dependent, non-transformed T cell line becomes independent of exogenous IL-2 and tumorigenic when it is infected by a retrovirus expressing the IL-2 gene.[116] Involvement of an IL-2/IL-2R-mediated autocrine mechanism has also been suggested in some non-ATL T cell malignancies.[117]

c. Interleukin-3

Interleukin-3 (IL-3), also called multi-CSF, is a 14–28 kd glycoprotein produced by activated T cells.[118, 119] The factor has effects on lymphoid, myeloid, erythroid and megakaryocyte growth by acting at an early stage in stem cell differentiation.[120] Both IL-3 and GM-CSF are secreted by activated T cells thereby inducing rapid local hematopoiesis, especially granulocytes and macrophages, in inflammatory tissues. As shown previously in the case of IL-2 and other factors, *in vitro* reconstitution of the IL-3 autocrine loop with IL-3-dependent cells resulted in factor-independent cells with tumorigenic activity when injected into nude mouse.[121] This observation may also suggest that dysregulated expression of the IL-3 gene is involved in leukemogenesis.

d. Interleukin-4

Interleukin-4 (IL-4), formerly called B cell stimulatory factor (BSF)-1, was originally identified by its ability to potentiate B cell proliferation.[122] Subsequent studies have shown that this molecule is also a growth factor for mast cells as well as some T cells.[123, 124] The relation of the factor to cellular malignancy has not yet been reported.

e. Interleukin-5

Interleukin-5 (IL-5), previously called T cell-replacing factor (TRF), was first defined to have a B cell differentiating activity.[125, 126] In addition, a recent study revealed that it is identical to eosinophil-differentiation factor.[127] The relation of this molecule to malignancy is also uncertain at present.

f. Interleukin-6

Interleukin-6 (IL-6), a glycoprotein of 184 amino acids, was originally identified as B cell stimulatory factor-2 (BSF-2),[128] interferon β-2[129] or a 26 kd protein.[130] This molecule manifests various biological effects on cells, including B and T cells, liver cells, neural cells and hematopoietic stem cells.[131, 132] Among those biological effects, it should be noted that IL-6 functions as a myeloma growth factor.[133] The observation that anti-IL-6 inhibits *in vitro* growth of myeloma (plasmacytoma) cell lines supports the idea that a dysregulated IL-6 autocrine loop is involved in a certain type of B cell malignancy.

8. 5q− Abnormality and Malignancy of Hematopoietic Cells

The 5q− syndrome is an established clinical entity characterized by a refractory anemia with a deletion of the long arm of human chromosome 5.[134] The bone marrow examination of the patients with this syndrome shows increased cellularity with decreased erythroid precursors as well as the presence of abnormal megakaryocytes. Those

findings are diagnosed as myelodysplastic syndrome defined according to the classification of acute myeloid leukemias proposed by the French-American-British (FAB) co-operative group.[135] Some patients with 5q− show an increase of myeloblasts (RAEB in the FAB subclassification) and develop acute myelocytic leukemia (AML)[136] and, in rare cases, multiple myeloma.[137] All patients who developed leukemias had further karyotypical abnormality other than 5q−.[136, 137]

The relationship of hematological malignancy and 5q− has also been reported. In adult AML, abnormal karyotypes were noted in 50 to 80% of patients and some of them (about 7%) show 5q− or monosomy 5.[138] The 5q− abnormality has also been reported in patients with polycythemia vera, chronic myelocytic leukemia, pure red cell aplasia and acute lymphocytic leukemia.

Much attention has been focused on 5q− syndrome since this chromosomal segment deleted in the patients (the deletion point is located between 5q12–5 and 5q31–33) has been reported to contain genes for various growth factors and receptors. Genes localized to 5q include those for hematological growth factors and receptors such as IL-3/multi-CSF (5q23–31),[139] CSF-1/M-CSF (5q33.1),[140] GM-CSF (5q21–32 or 5q23–31),[141] c-*fms*/CSF-1-R (5q33.2–33.3),[141] as well as the genes encoding PDGF-R (5q31–32),[16] β_2 adrenergic receptor (5q31–32)[142] and the glucocorticoid receptor (5q11–23).[143] Thus, genes encoding 3 of 4 CSFs are located on 5q and the gene for IL-3 is located within 10 kb of the GM-CSF gene. In contrast, the gene encoding G-CSF is located on chromosome 17q in man.[140]

The association of 5q− with hematological disorders may have profound significance. Although the mechanism by which deletion of one of two alleles located on 5q leads to hematological disorders still remains unclear, the loss of the critical region of 5q that leads to hemizygosity of a recessive allele might be involved in this process. Such a mechanism has been evoked in retinoblastoma.[144]

9. Oncogene Activation and Growth-factor Dependency

Evidence that activation of a non-growth factor/receptor type oncogene leads to the production of a growth factor that is directly involved in autocrine growth of the transformed cells has been provided. Chicken myeloid cells transformed by v-*myb* or v-*myc* are dependent on a chicken myelomonocytic growth factor (cMGF) for their *in vitro* growth.[145] Superinfection of retroviruses containing oncogenes belonging to the *src* gene family (those which encode non-receptor type tyrosine kinase) conferred on those cells growth factor independence through spontaneous production and consumption of a cMGF like factor in an autocrine fashion.[145]

On the other hand, expression of certain oncogenes often results in the abrogation of growth factor dependency of the cells. In the case of Abelson murine leukemia virus (Ab-MuLV) which is known to induce lymphoma of pre-B cell origin as well as other hematological malignancy, cellular transformation by v-*abl* does not depend on the autocrine mechanism. In fact, infection of growth factor (IL-2, IL-3 or GM-CSF)-dependent cells with Ab-MuLV resulted in the generation of factor-independent transformants, and they did not produce the growth factors required for cell growth prior to the transformation.[146–148] Similarly, expression of v-*myc*[149] or v-*src*[150] in IL-2- or IL-3-dependent cells resulted in the abrogation of the factor dependency for their growth.

Implications and Perspectives

Much of the information summarized above demonstrated that the genes encoding normal growth factors and receptors are in fact potential oncogenes. In order to understand the transforming process initiated by the aberrant expression of a growth factor and/or receptor at the molecular level, it is thus quite important to identify and characterize molecules involved in intracellular growth signal pathways. At present we do not know how many pathways exist in a single cell. It is likely that some of them are common to all cell types, while others are cell-lineage specific. In this context, the

observation that expression of viral oncogenes such as v-*abl*, v-*myc* or v-*myb* abolishes dependency on growth factors such as IL-2, IL-3 and GM-CSF, may suggest the existence of a common intracellular growth signal pathway(s) for the several cytokine/receptor systems in different cell lineages. This notion was further supported by the fact that the growth factor dependency of some pro-T cell clones can be switched from IL-3 to IL-2 following treatment with 5-aza-cytidine.[151] In addition, a recent study demonstrated that transfection and expression of EGF-R cDNA into a hematopoietic cell line whose growth is dependent on exogenous IL-3 resulted in the generation of cell clones that utilize EGF for their growth.[152] Here again, the result strongly suggests the existence of a common intracellular growth signal pathway(s) linked to the distinct growth factor-receptor systems.

At present, the initial cellular events mediated by the ligand-receptor interaction are still obscure. Several reports suggest that phosphatidylinositol (PI) turnover may be an indispensable process for the promotion of cell growth.[153] In fact, some growth factors such as PDGF and bombesin activate phospholipase C and promote PI turnover that is linked to Ca mobilization and protein kinase C activation.[154, 155] The activation of phospholipase C by ligand seems to be mediated via the GTP-binding proteins.[156] Thus, an attractive hypothesis is that such GTP-binding proteins couple growth factor receptors to the phospholipase C. In this regard, a *ras* gene product,[157] $p21^{ras}$, that is located on the inner surface of the cell membrane may also be a potential candidate as a receptor-associated molecule.[158] In fact, $p21^{ras}$ may be associated with receptors such as insulin receptor,[159] EGF-R,[160] bombesin receptor[161] and PDGF-R.[162] In addition, the fact that microinjection of *ras* protein into fibroblasts induces cellular proliferation suggests that the *ras* gene product is physically and/or functionally linked to the growth factor/receptor systems.[163]

We also have little information about the molecular process of growth signal transduction occurring between the cell membrane and the nucleus. Dysregulation of this process may also play a role in cellular transformation. This process probably includes non-receptor type tyrosine kinase molecules such as those encoded by *src* gene family (*src, yes, fgr, fyn, lyn, lck, hck*), *abl* and *fps/fes*, serine-threonine kinases (encoded by *raf* or *mos*) or C-kinases.

The growth signal triggered by interaction between growth factor and the receptor is finally transduced to the DNA replication machinery and/or RNA transcriptional machinery. Dysregulation of such machineries may be involved in cellular transformation.[164] This idea has been supported by a recent study which strongly suggests that the cellular counterpart of an oncogene v-*jun* is AP-1,[165] a DNA-binding protein that stimulates transcription of a specific set of genes by specifically binding to the regulatory region of those genes. The nuclear proto-oncogenes such as *myc, myb* and *fos* may also be involved in the regulation of genes whose products play a role in cell growth. Dysregulation of those genes may influence transcriptional programs required for normal cell proliferation. Studies such as molecular cloning of the genes encoding cellular transcription factors might uncover further the nature of oncogenesis.

Understanding the molecular mechanisms operating in growth signal transduction from membrane to nucleus (afferent loop) as well as from nucleus to cytoplasm and membrane (efferent loop) is one of the most important biological prerequisites for understanding cellular transformation, since it is quite evident that carcinogenesis is tightly linked to the disturbance of steps in the growth signal pathways.

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