JCI The Journal of Clinical Investigation

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J Clin Invest. 1984;74(3):669-676. https://doi.org/10.1172/JCI111482.

Research Article

Perspectives

Platelet-derived Growth Factor

Structure, Function, and Roles in Normal and Transformed Cells

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Polypeptide growth factors regulate the proliferation of cells in culture alone or in concert with other mitogens by inducing DNA synthesis and cell division in specific target cells. The mechanisms of action and the in vivo functions of these polypeptide growth factors are not known. It seems likely that growth factors have some role in cell development, differentiation, and tissue repair. Of the known growth factors, epidermal growth factor (EGF),[1] the platelet-derived growth factor (PDGF), and nerve growth factor are best defined. Each interacts with target cells through specific cell-surface receptors, leading to DNA synthesis, cell proliferation, and morphological and biochemical changes which resemble those characteristic of cells transformed by acute retroviruses (1–5). Malignant transformed cells also synthesize and secrete polypeptide growth factors which may stimulate autonomous cellular proliferation by an autocrine mechanism (6). In this article, we will summarize results of recent studies on the structure and biology of one well-characterized growth factor, PDGF, and attempt to relate how PDGF and perhaps PDGF-like molecules may play important roles in malignant transformation and cell growth of virus-transformed cells.

Origins of PDGF

Platelets do not bind to intact endothelium. PDGF is contained in alpha granules of platelets and is released only during blood clotting or when platelets adhere at sites of blood vessel injury. Secretion of platelet contents can be initiated by exposure of platelets to foreign surfaces such as subendothelial basement membrane or collagen (7–9). PDGF may serve to promote wound healing since it is the most potent mitogen in serum for cells of mesenchymal origins, including fibroblasts, glial cells, and smooth muscle cells (10, 11). Cells ordinarily are not exposed to serum; however, at sites of injury, platelets secrete PDGF, and blood coagulation reactions are initiated, providing locally in this instance a serum-like environment for cell growth.

Received for publication 1 May 1984.

1. *Abbreviations used in this paper:* EGF, epidermal growth factor; PGDF, platelet-derived growth factor; SSV, simian sarcoma virus.

J. Clin. Invest.

0021-9738/84/09/0669/08 $1.00
Volume 74, September 1984, 669–676

Physical/chemical characterizations of PDGF

PDGF was difficult to purify because of the very small quantities in platelets and because of contaminating proteolytic activity. Early purifications (12–14) succeeded in defining the properties of PDGF which were later exploited for larger scale purifications of milligram quantities of PDGF (15–19). PDGF is a highly basic (*p*I ~10.2) glycoprotein which has been separated into two equally active protein fractions differing only in covalently bound carbohydrate content. PDGF I is ~31 kD and contains ~7% carbohydrate, whereas PDGF II is ~28 kD and contains ~4% carbohydrate; PDGFs I and II have essentially equal mitogenic activity, amino acid composition, and immunological reactivity (15, 16, 20, 21). Both PDGF I and II appear to have 18 half-cystine residues all in disulfide linkage. Reduction of these disulfide linkages abolishes biological (mitogenic) activity and separates PDGF into larger A chains (PDGF I, ~17 kD; PDGF II, ~15 kD) and smaller B chains (PDGFs I and II, ~14 kD) (15, 16).

Anti-PDGF antisera have been used to measure PDGF levels in biological fluids by radioimmunoassay (21). Human serum contains ~50 ng/ml PDGF, whereas PDGF is not found in human plasma. A binding protein in plasma was observed that interferred with radioimmunoassay by specifically and covalently interacting with PDGF. This binding protein was later identified as alpha$_2$-macroglobulin (22). The physiological relevance of the binding of PDGF to alpha$_2$-macroglobulin is not known, but binding to alpha$_2$-macroglobulin when PDGF is released into serum may serve as a clearance mechanism to prevent biologically active PDGF from entering the systemic circulation from sites of injury.

Mitogenic responses to PDGF are mediated through binding to the PDGF receptor/protein tyrosine kinase

PDGF stimulates specific target cells by binding to cell surface receptors which, in turn, mediate a cascade of events that

leads to DNA synthesis and to cell proliferation. The PDGF receptor, as opposed to PDGF itself, may contain the requisite activity or sites required to initiate the proliferative response in target cells. The characteristics of the plasma membrane receptor for PDGF have been investigated by use of the ^{125}I-labeled growth factor (23–27). ^{125}I-PDGF binds to cells specifically and reversibly, with a K_d between 10^{-10} and 10^{-9} M. Approximately 400,000 binding sites per 3T3 cell have been found (23, 25). These binding parameters appear to reflect a physiologically reactive cell surface receptor, since the K_d of binding is similar to the half-maximum concentration required for the PDGF-dependent cytoplasmic phosphorylation of the ribosomal S6 protein in 3T3 cells (28) and for the PDGF-dependent stimulation of [^{3}H]thymidine incorporation into 3T3 cells (20). After ^{125}I-PDGF binds to the cell surface it is internalized and degraded. It is thus likely that PDGF, once bound to the cell surface, is internalized by vesiculation of coated pits and degraded in the lysosomes. The fate of the PDGF receptor is not understood. ^{125}I-tyrosine is released into the medium with a half-time of ~90 min at 37°C. At 0°C, cell-bound ^{125}I-PDGF is not degraded. Unlabeled PDGF, but not other growth factors, competes directly for binding with ^{125}I-PDGF. Protamine, a competitive inhibitor of ^{125}I-PDGF binding to the PDGF receptor, inhibits PDGF-stimulated [^{3}H]thymidine incorporation into 3T3 cells (25).

PDGF receptor binding activity is sharply reduced if cells are preincubated with PDGF. The number of binding sites for PDGF is reduced to ~10% of control values after cells are incubated with 200 ng/ml PDGF (25); this receptor down-regulation is observed with other growth factor receptors as well (29). The physiological significance of receptor down-regulation is not established, but down-regulation may serve as a mechanism to limit additional cellular proliferation that might result from prolonged exposure to growth factors.

An immediate consequence of growth factor binding to specific cell-surface receptors is protein phosphorylation. Tyrosine phosphorylation of EGF receptors can be demonstrated within 1–2 min of EGF addition to target cells (30, 31). The EGF cell surface receptor has been purified and shown to be a tyrosine-specific protein kinase; the EGF receptor-kinase itself can serve as a substrate for the EGF protein tyrosine kinase activity, i.e., autophosphorylation (32, 33). PDGF likewise stimulates tyrosine-specific protein kinase activity in membranes of fibroblasts (34, 35), phosphorylating a 180 kD protein thought to be the PDGF receptor. Recently, the receptor protein for PDGF has been purified and found to be a 180 kD glycoprotein with intrinsic and PDGF-stimulated protein tyrosine kinase activity (36). The PDGF receptor/kinase can also serve as substrate for its own kinase activity; the function of autophosphorylation of PDGF and other receptor proteins has yet to be elucidated but may be related to the regulation of receptor activity or turnover or may be directly responsible for initiating the biochemical events that lead to cell division.

Tyrosine kinase activity in viral transformed cells

The tyrosine-specific protein kinase activity of the PDGF receptor protein suggests a close parallel to biological activities observed in retrovirus transformed cells. The transforming protein of the acute transforming Rous sarcoma virus (37), pp60$^{v\text{-}src}$, is a tyrosine-specific protein kinase and is responsible for the initiation and maintenance of transformation in Rous sarcoma virus infected cells. The pp60$^{v\text{-}src}$ protein kinase activity is the product of the viral oncogene v-src and is believed to be active in transformed cells because Rous sarcoma virus transformed cells have increased levels of phosphotyrosine residues in proteins compared with the levels in nontransformed cells (37–42). The association of viral oncogene-protein tyrosine kinase activity and growth factor receptor-protein tyrosine kinase activity has suggested that the activation of these kinases may initiate a common cascade of growth regulatory pathways in transformed cells and in cells stimulated by growth factors that lead to cellular proliferation.

The normal cellular genomic homologue of v-src (the proto-oncogene, c-src) has been found in normal eukaryotic cell DNA (43–47); this finding suggested that the viral oncogene was acquired from normal cellular chromosomal DNA by genetic recombination, a suggestion confirmed in later studies. Phosphotyrosine normally is present in eukaryotic cellular proteins in relatively small amounts, accounting for ~0.01–0.03% of total amino acids in proteins (48). Attempts have been made to identify the naturally occurring protein substrates for the tyrosine kinase activity associated with Rous sarcoma virus transformation and growth factor stimulation of cells. Whereas a number of common substrates for protein tyrosine kinases have been identified (49, 50), the true physiological role of tyrosine kinases and autophosphorylation in growth factor-stimulated, viral-transformed cells has yet to be established.

Early activities of PDGF-stimulated cells

PDGF induces pleiotrophic effects when added to cultured fibroblasts or glial cells that contain PDGF receptors. Activities stimulated in target cells include the enhancement of glycolysis (51), the stimulation of amino acid transport and protein synthesis (52), the induction of early changes in phosphatidyl inositol and arachidonic acid metabolism (53), the stimulation of prostaglandin synthesis (54, 55), the stimulation of polysome formation (56), and the reorganization of actin filaments (as reviewed, 2, 3, 5; 57, 58). Of particular interest is the similarity of stimulated activities of cells exposed to growth factors and of cells transformed by viruses. Cells exposed to growth factors have increased levels of active transport, protein synthesis, etc., which are similar to those of viral and other transformed cells; this similarity, coupled with the ability of transformed cells to bypass the serum requirement for cell growth (59, 60), has suggested a close similarity in the mechanism of stimulation of biological activities in growth factor-stimulated and virus-transformed cells.

PDGF alone does not optimally stimulate DNA synthesis; PDGF-stimulated cells require plasma or an additional activity to initiate DNA synthesis and to divide (11, 61–64), suggesting that PDGF and plasma factors control different events in the cell cycle. PDGF is believed to make cells competent to enter the cell cycle and subsequently able to respond to factors in plasma in order to undergo progression from G_0/G_1 to enter S phase (65). Purified somatomedin C increases the number of PDGF-stimulated cells that enter the S phase but does not alter the 12-h lag period for G_0/G_1 progression. Somatomedin C has been identified as a progression factor that is normally present in plasma. Competence can be transferred from PDGF-triggered donor Balb/c 3T3 cells to untreated recipients by cell fusion (66); the treatment of donor cells with inhibitors of RNA synthesis blocks the ability of these cells to provide the cytoplasmic signal. PDGF-inducible gene sequences have been identified in low-abundance messenger RNA (mRNA), which increases ~10-fold after PDGF stimulation. The inducible mRNAs appear to correlate in time sequence with the selective synthesis of cytoplasmic proteins from previously quiescent Balb/c 3T3 cells (58, 67).

Chemotactic activity: possible normal role of PDGF in wound healing

PDGF is generally believed to be the principle mitogen that stimulates cell division where vessel integrity has been compromised and platelet activation has occurred. PDGF has a second property, however, which suggests further that a normal role of PDGF may be to act as a mediator of inflammation and repair; PDGF is a potent chemotactic protein for inflammatory cells. Maximum neutrophil chemotaxis occurs at PDGF concentrations of 1–5 ng/ml, whereas PDGF-stimulated monocyte chemotaxis occurs maximally at a concentration of 20 ng/ml (68). These concentrations of PDGF are well below those found in human serum (50 ng/ml) (21). At somewhat higher concentrations (20–40 ng/ml), PDGF activates human neutrophils, initiating superoxide synthesis and release, specific neutrophil granule release, and neutrophil aggregation (Tseng, D., T. F. Deuel, J. Huang, L. Boxer, R. Senior, and R. Baehner, unpublished data). Smooth muscle cells and fibroblasts are similarly strongly attracted to low concentrations of PDGF (10–20 ng/ml) (69–71), suggesting these cells may migrate to injured sites where subsequent mitogenic stimulation furthers repair processes. PDGF-stimulated fibroblasts synthesize collagenase; PDGF at 20 ng/ml increases the synthesis and release of collagenase from cultured human skin fibroblasts approximately sevenfold (Bauer, E., J. S. Huang, T. F. Deuel, J. Altman, and J. Cooper, unpublished data), perhaps serving a vital function in hydrolyzing damaged collagen before wound remodeling and repair. The structural determinant of PDGF that mediates chemotaxis may be different from the one that mediates mitogenesis (72).

The presence of inflammatory cells and fibroblasts in wounds (and smooth muscle cells in wounds of blood vessels) may be correlated with the PDGF concentrations achieved by platelet release. In the guinea pig, fibrin formation is first found in the healing wound; neutrophil influx is then followed by macrophage influx and then by fibroblast migration (73). The temporal migration of these cells into wounds thus correlates reasonably well with concentrations of PDGF required for maximum chemotaxis. At the higher concentrations of PDGF believed to be present more proximally in wounded areas into which cell migration has occurred, neutrophil activation, fibroblast release of collagenase, and cell division follow. PDGF thus may play a pivotal role in wound healing (Fig. 1); this role may be the primary function of PDGF in vertebrates.

Structural/functional homology of PDGF and the putative transforming protein of the simian sarcoma virus

Normal vertebrate cells contain highly conserved cellular genes called proto-oncogenes which, if inappropriately expressed, produce transforming proteins that can induce and maintain malignant cell growth. When incorporated into the genome of acute transforming retroviruses, these transduced cellular sequences (oncogenes) acquire the ability to induce neoplastic transformation. Normal cellular proto-oncogenes may be converted to transforming genes by other mechanisms including point mutations, gene rearrangements, gene translocations, and gene amplification (as reviewed, 74). As noted above, several lines of evidence indicated a similarity of cellular alterations induced by activated transforming proteins and by the growth promoting activities of polypeptide growth factors, suggesting a relatedness of growth factor activity and transforming protein activity.

Direct support for such relatedness was recently obtained when the amino acid sequence analysis of PDGF resulted in the remarkable finding of >90% homology between the amino acid sequence of the larger chain (A chain) of PDGF and the predicted amino acid sequence of $p28^{v\text{-}sis}$, the putative transforming protein of the simian sarcoma virus (SSV) (75, 76) (Fig. 2). Subsequent sequence analysis coupled with the isolation and sequencing of human c-sis genomic DNA (77) established the nucleotide sequences of five regions of the human c-sis gene that were homologous to sequences of the transforming region (v-sis) of SSV. Analysis of the A chain of PDGF showed identity of amino acid sequences predicted for the c-sis gene product through 109 residues. Thus, the human c-sis gene almost certainly encodes a polypeptide precursor of the A chain of PDGF; a partial sequence of the smaller chain (B chain) is 60% homologous to the A chain and cannot be encoded by that part of the c-sis genomic DNA so far sequenced, but it may lie 5′ to the 5 regions of v-sis homology in c-sis or may be encoded by a separate locus.

This striking amino acid sequence homology between PDGF and $p28^{v\text{-}sis}$ suggested that cells transformed by SSV produce a transforming protein highly similar to PDGF not only in structure but in mitogenic activity as well. This

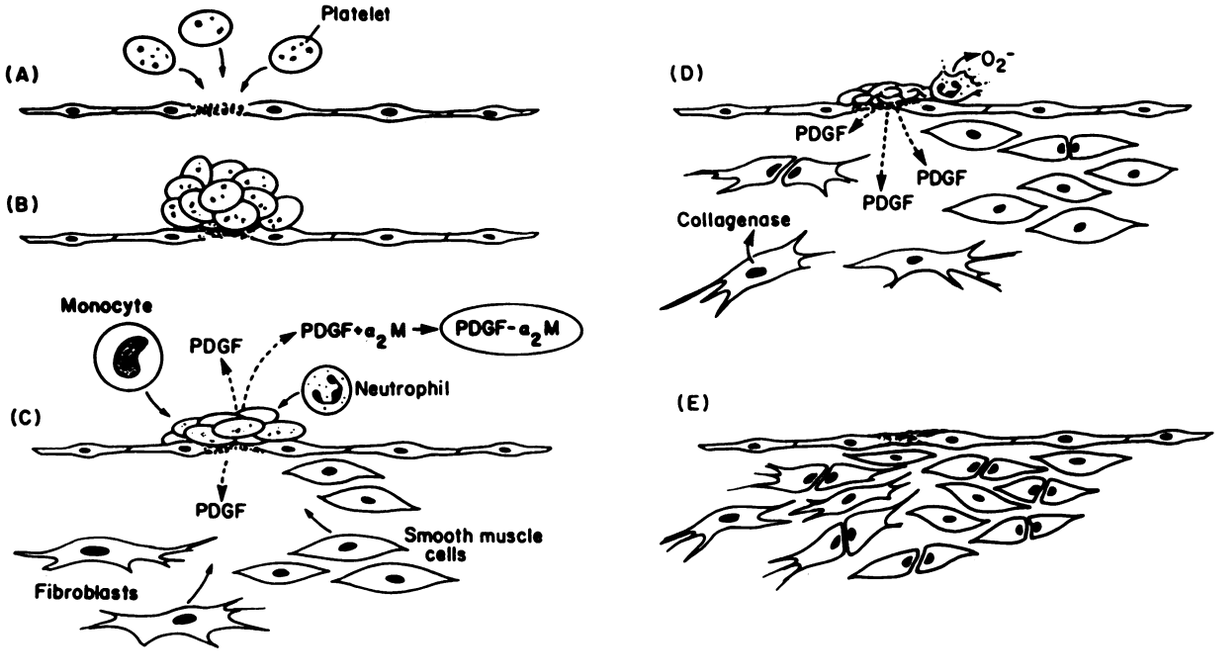

Figure 1. Potential roles of PDGF in wound healing. PDGF is released after injury (*A*) and platelet adherence and aggregation at injured sites (*B*). The PDGF released locally stimulates the migration of neutrophils, monocytes, fibroblasts, and smooth muscle cells (in vessel wall injury) into the wounded sites; PDGF released into the systemic circulation is complexed to alpha$_2$-macroglobulin (α_2M) (*C*). At the higher concentrations of PDGF proximal in the wound, PDGF activates neutrophils to generate and release superoxide and stimulates fibroblasts to synthesize and release collagenase. PDGF also stimulates cellular proliferation of fibroblasts and smooth muscle cells (*D*). Remodeling and reorganization follow (*E*).

mitogenic activity has now been identified in SSV-transformed National Institutes of Health (NIH) 3T3 cells and has been shown to be essentially identical to PDGF in mitogenic dose response curves, in specific mitogenic activity, and in reactivity in immunoassays (78). Immunoprecipitates with anti-PDGF antisera of cell lysates from SSV-transformed NIH 3T3 cells contained a protein of 20 kD, consistent with the proteolytically processed product of p28$^{v\text{-}sis}$ (78). Thus, the protein in SSV-

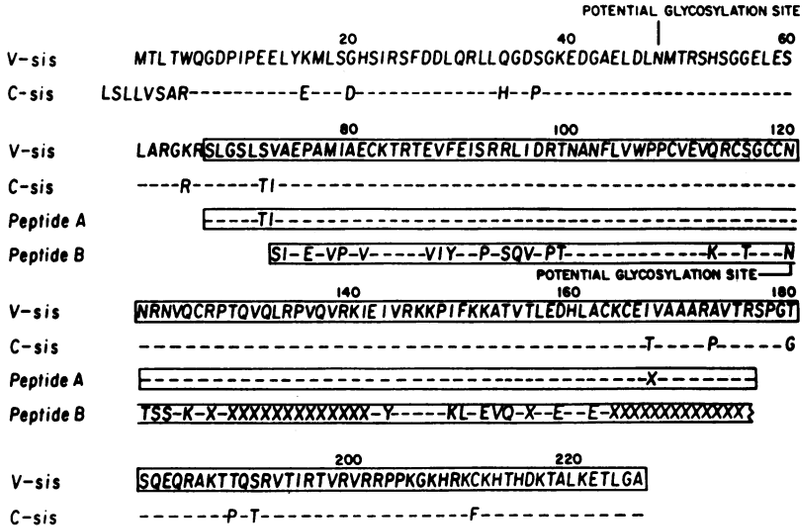

Figure 2. Comparison of amino acid sequences of the proteins predicted from the c-sis and v-sis nucleotide sequences with the sequences of the A and B chains of human PDGF. The sequences of the A and B chains were provided from structural analysis of high-performance liquid chromatography-fractionated peptides (75). The predicted amino acid sequence of c-sis and figure display have been adapted from Johnsson et al. (77). Bars indicate identity to the amino acid residue predicted from the v-sis gene (103), and X represents amino acid residues that have not been identified with certainty. The numbering of residues begins at the amino terminus of v-sis. Amino acid residues are expressed in the single letter code: A (Ala), C (Cys), D (Asp), E (Glu), F (Phe), G (Gly), H (His), I (Ile), K (Lys), L (Leu), M (Met), N (Asn), P (Pro), Q (Gln), R (Arg), S (Ser), T (Thr), V (Val), W (Trp), and Y (Tyr).

transformed cell lysates with growth promoting activity related to PDGF is almost certainly $p28^{v\text{-}sis}$; this strongly supports the idea of a role of PDGF-like mitogenic activity as a mediator of virus transformation in SSV-transformed cells. SSV-transformed cells also secrete a PDGF-like growth promoting activity into conditioned media; this secreted protein also has been identified as $p28^{v\text{-}sis}$ or its cellular processed product (Huang, J. S., S. S. Huang, and T. F. Deuel, unpublished data). The addition of antisera to cultures of growing SSV-transformed NIH 3T3 cells progressively reduced the incorporation of [^{3}H]thymidine into DNA as concentrations of antisera were increased, presumably by interacting with secreted $p28^{v\text{-}sis}$ and blocking the $p28^{v\text{-}sis}$ feedback stimulation of [^{3}H]thymidine incorporation through receptors on the surface of the transformed cells. SSV-transformed NIH 3T3 cells respond mitogenically to PDGF and have a limited number (~5%) of ^{125}I-PDGF receptors compared with that of non-transformed cells. These receptors appear to recognize $p28^{v\text{-}sis}$ because partially purified $p28^{v\text{-}sis}$ competed with ^{125}I-PDGF for binding. Thus, the evidence suggests that the transforming protein $p28^{v\text{-}sis}$ has potent growth factor activity; $p28^{v\text{-}sis}$ is expressed and secreted from SSV-transformed cells and then appears to stimulate the secreting SSV-transformed cells by interacting through common receptors that recognize $p28^{v\text{-}sis}$ and PDGF. $p28^{v\text{-}sis}$ thus serves as an autocrine regulator of SSV-transformed cell growth. The SSV-transformed NP1 cell line (marmoset fibroblasts) does not secrete a protein with growth promoting activity into conditioned media within detectable limits of the assay, suggesting that transformation per se, as opposed to autocrine stimulation of cell growth, may not require secretion of the transforming protein but may be mediated through the intracellular recognition of $p28^{v\text{-}sis}$. Expression of the transforming activity of $p28^{v\text{-}sis}$ may therefore occur after the release of $p28^{v\text{-}sis}$ from ribosomes, perhaps within the endoplasmic reticulum as the recognition site of the newly synthesized receptor protein (amino terminus) or another receptor enters the endoplasmic reticulum, with the kinase activity remaining outside free within the cytoplasm.

In SSV-transformed 3T3 cells, $p28^{v\text{-}sis}$ is a glycosylated protein which is subsequently processed proteolytically to generate disulfide-linked dimers structurally similar to PDGF (79). Proteins with structural and functional similarities to PDGF have been identified in conditioned media of human osteosarcoma and glioma derived cells lines (80–82) and in an SV40-transformed baby hamster kidney cell line (83–85), suggesting that activation of genes encoding PDGF or a closely related protein may be important in transformation by other viruses or in non–viral-transformed cells. Such may be the case with the HUT-102 cell line, derived from a cutaneous T cell lymphoma and infected with the human T cell leukemia virus. This cell line expresses c-sis and thus is unusual among hematopoietic cell lines (86–88). mRNA transcripts of the human proto-oncogene c-sis have been detected with v-sis probes in glioblastoma and sarcoma cell lines but not in cell lines derived from normal fibroblasts or from melanoma or carcinoma cell lines (89). The c-sis gene product alone appears to be required for transformation; a complementary DNA (cDNA) clone from HUT-102 cells transforms NIH 3T3 cells, but since the sequence of the cloned cDNA was not reported, it is not yet known whether the ability of this cDNA to transform 3T3 cells is due to a mutation (90).

The transforming sis gene protein products, although not yet adequately characterized by direct physical measurement, appear to be homodimers of v-sis or c-sis. The role of the less homologous PDGF B chain remains to be elucidated. The human proto-oncogene c-sis has been mapped to a locus on chromosome 22 (91, 92). Deletions of the long arm of human chromosome 22 have been observed in human meningiomas (93, 94); nonrandom rearrangements of human chromosome 22 are found in human hematopoietic malignancies; and in chronic myelogenous leukemia, a segment of the long arm of chromosome 22 is translocated to chromosome 9 (95). Likewise, chromosomal translocations of band 22 have been found in Ewing's sarcoma; these cell lines fail to show detectable sis polyadenylated mRNA (96, 97).

Future directions

Major questions have been raised and many new lines of research are suggested by the observations discussed. It seems important, for example, to understand the relationship of the heterodimer (A and B chains) structure of PDGF in comparison with the apparent homodimer construction of v-sis and c-sis expressed gene products. Expression of the A chain alone in dimeric structure appears to be able to induce cell proliferation and/or cell transformation. The function and genomic locus of the B chain in PDGF is not known; however, the B chain may act similarly to the A chain, or, conversely, it may regulate A chain activity, thereby limiting PDGF to a mitogenic, as opposed to a transforming, stimulus. The physiological role of PDGF, apart from its proposed role in wound healing, is not yet known. Evidence in favor of a role of growth factors/oncogene products in differentiation and development is slowly accumulating (98), consistent with the highly conserved nature of oncogenes in evolution. Interactions with other oncogenes are likely; for example, PDGF initiates a 10–40-fold increase in mRNA concentration of the proto-oncogene c-myc (99). The role of growth factors in general in malignant transformation also needs further investigation to determine if the role of PDGF-like proteins in transformation is an isolated one. The secretion of transforming growth factors by cultured sarcoma cells, however, argues strongly for a more general role of growth factors in malignant transformation.

Oncogenes may be derived from normal cellular genomic sequences encoding for growth factor receptors and produce transformation through the unregulated expression of growth factor-receptor activity. The role of the PDGF receptor protein needs to be established to indicate whether the receptor protein contains the information required for cellular proliferation

and/or malignant transformation. The role of the receptor protein tyrosine kinase activity must also be determined in order to understand whether it is required for cell proliferation/transformation or is important in regulation of receptor endocytosis or agonist binding. That the PDGF receptor also has the potential to function as an oncogene product (transforming protein) has been established with near certainty with the recent recognition that six peptides derived from the human EGF receptor protein match closely the deduced amino acid sequence of the v-erb-B transforming protein of the avian myeloblastosis virus (100). Thus, the receptor protein for EGF and presumably for PDGF appears to have the potential to stimulate both cellular proliferation and transformation.

How $p28^{v\text{-}sis}$ initiates transformation also must be understood. The apparent failure of some SSV-transformed cells to secrete transforming protein implies strongly that malignant transformation in SSV-transformed cells can be mediated through the intracellular recognition of $p28^{v\text{-}sis}$, as opposed to growth stimulation by the autocrine secretion of transforming protein. A possible mechanism whereby this might occur is when newly synthesized PDGF receptor protein with the PDGF recognition site within the endoplasmic reticulum interacts with $p28^{v\text{-}sis}$. In this circumstance, activated receptor protein tyrosine kinase activity would remain exposed to the cytoplasmic surface and perhaps initiate a cascade of reactions to initiate the inappropriate cell division responsible for transformation.

The role of growth factors in normal cellular physiology and in malignant transformation remains elusive, but provocative results now suggest the relationships soon will be better understood. The results are likely to suggest complex mechanisms of tumor formation; carcinogenesis appears to involve multiple steps (101), and multiple cellular oncogenes seem to be transcriptionally active in tumors (102).

Acknowledgments

The authors gratefully acknowledge many helpful comments during the course of this work by Drs. Luis Glaser and John Rogers, and many other colleagues, and a critical reading and commentary of the manuscript by Drs. Philip Majerus, Timothy Ley, and Benton Tong.

This work was supported by grants awarded by the National Institutes of Health (CA 22409, HL 14147, and HL 22119 to T. F. Deuel), the American Heart Association, Missouri Affiliate, Inc. (Grant-in-Aid to J. S. Huang), and the Monsanto Co., St. Louis, MO.

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