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The Role of Polypeptide Growth Factors in Human Carcinomas: New Targets for a Novel Pharmacological Approach

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***Abstract*——The processes of cellular proliferation and progressive acquisition of a specialized phenotype show a high degree of coordination. In particular, these complex signaling networks mediating cell growth, differentiation, migration, and apoptosis are regulated in part by polypeptide growth factors that can act, by autocrine and/or paracrine mechanisms of action, as positive or negative modulators. Because these growth factors are unable to cross the hydrophobic cell membrane, they exert their effects via binding to cell surface receptors, most of which possess intrinsic tyrosine kinase activity. Owing to the interaction of polypeptide growth factors with their specific transmembrane receptors, a cascade of intracellular biochemical signals, resulting in the activation and repression of various subsets of genes, is triggered. One of the major incentives for studying factors that**

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regulate processes of proliferation and differentiation is the recognition of their involvement in tumorigenesis. Genetic aberrations in growth factors signaling pathways are, in fact, inextricably linked to cancer. Malignant cells arise as a result of a stepwise progression of genetic events characterized by the unregulated expression of growth factors or components of their signaling networks. The main aim of this review is to examine the current understanding of the crucial contribution that several growth factors may have on transformation, tumorigenesis, and progression in several human tumors among the most widespread in western countries. For this purpose, we will analyze the chemistry and the molecular organization of the most important growth factors and their specific receptors. In addition, we will focus on the mechanisms of signal transduction, the complex cascade of biochemical events ensued from the growth factor/receptor binding. The present knowledge of the role of growth factor biochemical signaling networks in cancer leads to improvements not only in diagnosis and prognosis for this disease, but also for new and more targeted therapeutic intervention. The second part of this review will focus on the novel pharmacological approaches for cancer therapy that have been developed already or are being developed with the aim to specifically interfere at various steps of the growth factors signaling pathways.

I. Background and Rationale

A. Generalities on Growth Factors and Receptors

In multicellular organisms, cellular interactions are controlled by highly coordinated mechanisms. These complex networks are also responsible for responses to infection and wounding, and likewise mediate normal embryonic development. Since the discovery of nerve growth factor (Levi-Montalcini, 1987) and epidermal growth factor (EGF)[2] (Cohen, 1986), a wide array of polypeptides involved in the modulation of cell proliferation have been identified. Such growth-controlling molecules influence cell growth and differentiation both positively and negatively. Growth factors may have either a paracrine (i.e., released by one cell type and acting on another cell) or autocrine (i.e., acting on the same cell by which it has been secreted) influence on cell proliferation. In addition, the ultimate response of a target cell to a particular growth factor is determined by the cellular context in which the stimulus is received, such that some factors are able to give rise to qualitatively different responses. This occurs during the myeloid differentiation of hemopoietic cells, whose response changes from proliferation to priming in precursor and mature cells, respectively (Cross and Dexter, 1991). However, qualitatively different results may even arise in a concentration-dependent manner from the action of a growth factor on a specific cell type (Cross and Dexter, 1991).

The activation and/or repression of a subset of genes shown to function at critical steps in mitogenic stimulus is the result of the cascade of intracellular biochemical signals ensuing from the interaction of growth factor with specific receptors. Because these growth factors are unable to cross the hydrophobic cell membrane, a fundamental question is how they transduce their signals into the cells. Growth factors exert their effects via binding to cell membrane receptors, and it has been shown in recent years that these receptors are often activated by ligand-induced dimerization or oligomerization (Heldin, 1995, 1996). Receptor dimerization is in some cases the result of the interaction between a symmetrical, dimeric ligand which binds to two receptor molecules (Heldin, 1996) (Fig. 1). On the contrary, other ligands, such as members of the EGF-family, are apparently monomers. It seems, however, that two ligands interact with two EGF-receptors in a symmetrical fashion (Lemmon and Schlessinger, 1994). Another possibility for dimerization is exemplified by basic fibroblast growth factor that binds to a high- and a low-affinity receptor.

Many traditional growth factors (e.g., EGF, fibroblast growth factors, and insulin-like growth factors) bind to receptors with protein tyrosine kinase (PTK) activity. All PTK receptors (PTK-R) consist of single transmembrane domains that separate the intracellular kinase domains from the extracellular portions. These latter domains contain one or several copies of Ig-like, EGF-like, or fibronectin type III-like domains (Heldin, 1995). The catalytic (kinase) domains display the highest level of conservation. Structural motifs that are conserved in this region include an ATP-binding site and a tyrosine residue, which corresponds to the major phosphate acceptor site (Yarden and Ullrich, 1988).

B. Signal Transduction Pathway

The mechanism of ligand-induced dimerization of PTK-R, whereby the receptors are activated, involves

[2] Abbreviations: EGF, epidermal growth factor; EGF-R, EGF receptor; PTK, protein tyrosine kinases; PTK-R, PTK receptor; SH2, Src-homology 2; PTB, phosphotyrosine binding; PLC, phospholipase C; PI3K phosphatidylinositol-3′-kinase; Grb2, growth factor receptor binding; SH3, Src-homology 3; sos; son of sevenless; MAP, mitogen-activated protein; MEK, MAP kinase kinase; PTP, protein tyrosine phosphatase; PTPases, phosphotyrosine phosphatases; TGF-α, transforming growth factor-α; STATs, signal transducers and activators of transcriptions; TAF, transcription activator factor; FGF, fibroblast growth factor; aFGF, acidic FGF; bFGF, basic FGF; PI, phosphoinositol; IGF, insulin-like growth factor; IGF-R, IGF receptor; hr-IGF, human recombinant-IGF; IRS, insulin receptor substrate; BP binding protein; VEGF, vascular endothelial growth factor; SCLC, small cell lung cancer; N-SCLC, non-small cell lung cancer; TGF-β, transforming growth factor-β; HGF, hepatocyte growth factor; SF, scatter factor; PDGF, platelet-derived growth factor; PDGF-R, PDGF receptor; PPS, pentosan polysulfate; MoAb, monoclonal antibody; 4-HPR, *N*-(4hydroxyphenyl)retinamide; FTIs, farnesyl transferase inhibitors; RhoB-F, farnesylated form of RhoB; RhoB-GG, geranylgeranylated form of RhoB.

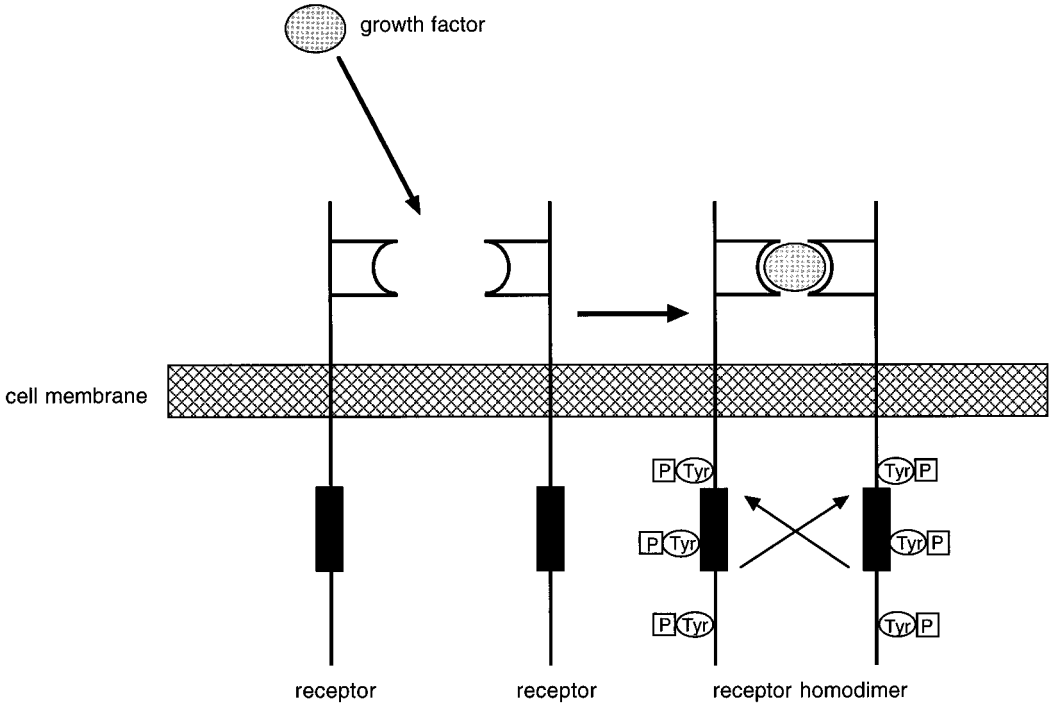


FIG. 1. Ligand-induced receptor homodimerization triggering the initial step of cell signaling pathways. The juxtaposition of the cytoplasmic regions of the receptors makes it possible for the kinase domains (■) to phosphorylate (open square containing P) each other at the tyrosine residues (open oval containing Tyr). The positions of phosphorylated tyrosines, which serve as docking sites for adaptor molecules, are schematically represented.

juxtaposition of the cytoplasmic parts of the receptors that allows the kinase domains to phosphorylate each other (Fig. 1). Autophosphorylation involves two different classes of tyrosine residues. One phosphorylation occurs on a conserved tyrosine residue within the intracellular kinase domain. It is still not completely clear how autophosphorylation is initiated; one possibility is that the monomeric receptor has a low enough basal kinase activity to phosphorylate and activate the companion receptor resulting from dimerization. This would then be followed by reciprocal phosphorylation. Otherwise, the interaction between the intracellular domains of the receptors in the dimer may induce conformational changes leading to an increased kinase activity. The secondary autophosphorylation sites are normally located outside the kinase domains and serve the fundamental function of creating docking sites for downstream signal transduction molecules containing Src-homology 2 (SH2) domains or phosphotyrosine binding (PTB) tyrosine domains. The SH2 domain is a 100-amino acid motif that folds to form a surface that recognizes phosphotyrosine and three to six C-terminal amino acid residues (Pawson, 1995). The PTB domain has been identified in the amino terminus of Shc; this adaptor protein, also containing SH2 domains, appears to be involved in *ras* activation. PTB domains are longer than SH2 domains. In contrast to SH2 domains, they recognize phosphotyrosine in the context of an *N*-terminal residue (van der Geer and Pawson, 1995). Other SH2-containing molecules include phospholipase C (PLC), phosphatidylinositol-3′-kinase (PI3K), p21*ras* GTPase-activating protein, and growth factor receptor binding 2 (Grb2) protein.

PI3K is a dimer of a regulatory component of 85 kDa and a catalytic component of 110 kDa which phosphorylates phosphoinositides on the D-3 position. The reaction products can act on multiple downstream effectors that include SH2 and Pleckstrin homology domains of serine/threonine and tyrosine kinases and various cytoskeletal proteins (Carpenter and Cantley, 1996). It has been shown that a signaling pathway from PI3K to the serine/threonine protein kinase Akt/PKB may mediate some cellular responses of PI3K (Burgering and Coffer, 1995; Cross et al., 1995; Franke et al., 1995), including protection from apoptosis (Dudek et al., 1997; Franke et al., 1997). Although the exact mechanisms by which Akt/PKB prevents apoptosis are not completely highlighted, it has been proposed that Akt/PKB might mediate cell survival by phosphorylation and inhibition of proteins that are involved in programmed cell death.

Grb2 is an adaptor protein containing another type of domain, Src-homology 3 (SH3), which binds specific proline-rich motifs. These sites interact with a molecule known as *sos* (son of sevenless), which in turn activates *ras*. A signaling cascade through a series of kinases is then initiated; *ras* activates *raf-1*, which in turn activates mitogen-activated protein (MAP) kinase kinase (MEK); MEK activates MAP kinase, which translocates in the nucleus and activates transcription factors *myc*, *jun*, and *fos*. Interaction between *fos* and *jun* leads to the tran-

scription complex activator protein-1, which switches on a number of genes associated with cell growth and differentiation, as well as with the regulation of cell shape and chemotaxis (Yarden and Ullrich, 1988; Heldin, 1995, 1996; Langdon and Smyth, 1995) (Fig. 2).

The activation of signal transducers and activators of transcription (STATs) family members, which are latent cytoplasmic transcription factors, is an important way whereby PTK-R transduce their signal. Phosphorylation of a tyrosine residue conserved in all STATs family members induces their dimerization, which is followed by translocation into the nucleus, DNA binding, and regulation of numerous genes involved in growth and differentiation, such as $p21^{WAF1}$ (Ruff-Jamison et al., 1995; Chin et al., 1996; Xie et al., 1997) and c-fos (Sadowski et al., 1993).

After ligand binding and activation, PTK-R are deactivated by protein tyrosine phosphatases (PTP) that dephosphorylate the autophosphorylated tyrosine residues (Hunter, 1995). Several such PTP have been identified, and some of them have SH2 domains and bind to PTK-R. Dephosphorylation does not represent the only mechanism by which PTK-R are deactivated; receptors are also internalized in endosomes after assembly in coated pits. At this stage, ligands dissociate from the receptors because of the low pH and the receptor is either recycled back to the cell surface or degraded after the endosomes have fused with lisosomes (Heldin, 1996).

Dimerization has also been shown to occur after binding to serine/threonine kinase receptors (Heldin, 1995). Transforming growth factor β represents a prototype for a large family of structurally related factors that interact with such receptors. Ligand binding induces a hetero-oligomeric complex of type I and type II receptors, most likely a heterotetramer made up of two receptors of each type (Yamashita et al., 1994a). Type II receptor, which has a constitutively active kinase, first binds the growth factor. Type I is then recruited and the serine residues in the glycine-serine residues domain are phos-

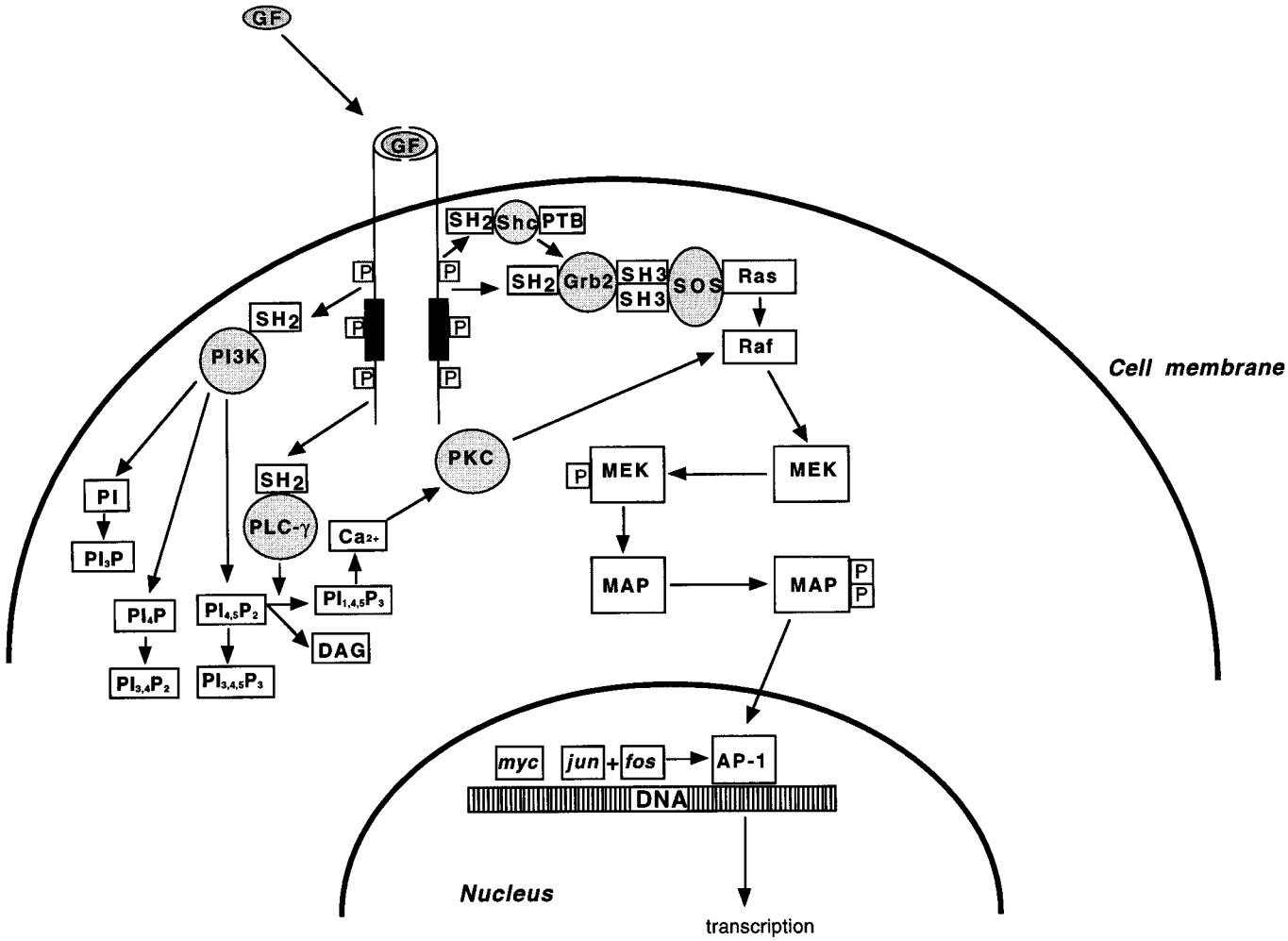


FIG. 2. Schematic representation of the cell signaling pathway mediated by growth factor (GF) receptors with intrinsic tyrosine kinase activity. AP-1, activator protein 1; DAG, diacylglycerol; MAP, mitogen activated protein kinase; MEK, MAP kinase kinase; P, phosphated region; PLC-γ, phospholipase C-γ; PI_3P, phosphatidylinositol(3)-phosphate; PI_4P, phosphatidylinositol(4)-phosphate; $PI_{3,4}P_2$, phosphatidylinositol(3,4,)-bisphosphate; $PI_{4,5}P_2$, phosphatidylinositol(4,5)-bisphosphate; $PI_{1,4,5}P_3$, phosphatidylinositol(1,4,5)-triphosphate; $PI_{3,4,5}P_3$, phosphatidylinositol(3,4,5)-triphosphate; PKC, protein kinase C; PTB, PTB domain; SH2, SH2 domain; SH3, SH3 domain.

phorylated, thereby triggering the signal transduction pathway (Heldin, 1995).

C. Cancer and Altered Growth Factor Signaling

Genetic aberrations in growth factor signaling pathways are strongly connected with developmental abnormalities and a variety of chronic diseases, chief of which is cancer. Tumor evolution is a multistep process requiring, first, that the normally interdependent systems controlling proliferation and differentiation are separated and, second, that proliferation is stimulated in such a way as to result in extensive growth of the transformed cells. Malignant cells arise either from alterations that may take the form of up- or down-regulation of growth factors and/or their receptors, or from a switch from a paracrine to an autocrine mechanism of action. Alternatively, given that many growth factors use a common signal transduction pathway leading to an intracellular biochemical cascade, any mutation in these cascades may affect several growth factor pathways simultaneously. Moreover, some growth factors have the ability to induce the extension of nearby blood vessels and, hence, have the potential to contribute to tumor vascularization. In the absence of vessels, proliferation at the surface of the tumor is balanced by cell death in the center. Once such a tumor begins to release angiogenic factors, the consequent vascularization allows cancer cells to spread through solid tissues (Blood and Zetter, 1990).

In the first part of this review, we describe the growth factor families involved in the most important carcinomas in humans; for this purpose, we analyze in detail the chemistry and molecular organization of the most common growth factors and their specific receptors. The complex cascade of biochemical events ensuing from growth factor/receptor binding is also discussed. We believe that the present knowledge of the role of growth factor biochemical signaling networks in cancer is pivotal to improvements not only in diagnosis and prognosis of this disease, but also in new and more targeted therapeutic intervention. Thus, the second part of this review focuses on the novel pharmacological approaches for cancer therapy that have already been or are being developed with the aim to specifically interfere at various steps of growth factor signaling pathways.

II. Growth Factor Families

A. Epidermal Growth Factor and Receptor

Human epidermal growth factor (EGF) (Table 1) is a 53-amino acid single-chain polypeptide (6 kDa) that arises from the proteolytic cleavage of a large (1207 amino acids) integral membrane protein precursor (Carpenter and Cohen, 1990). This precursor protein is composed of eight extracellular EGF-like domains, only one of which has EGF-like activity. This growth factor, whose gene is positioned on human chromosome 4, stimulates the proliferation of epithelial cells, inhibits gastric acid secretion, and is involved in wound healing. EGF is closely related structurally to transforming growth factor-α and to vaccinia growth factor, both of which bind to EGF-receptor (EGF-R).

TABLE 1
Main characteristics of polypeptide growth factors and their receptors

Ligand	Length	MW	Human Chromosomal Location	Receptor	Length	MW	Human Chromosomal Location
	aa	*kDa*			*aa*	*kDa*	
EGF	53	6	4	EGF-R	1186	170	7
aFGF	115	16	5	FGF-R-1	801	160	8
bFGF	155	16–18	4	FGF-R-2	800	135	10
				FGF-R-3	784	135	4
				FGF-R-4	778	140	5
IGF-I	70	7.6	12	IGF-I-R	706 (α) 196 (β)	130 (α) 90 (β)	15
IGF-II	60	7.4	11	IGF-II-R		260	6
TGF-α	50	6	2	EGF-R	1186	170	7
TGF-β_1	112	25	19	TGF-R-β_1	479	50–60	unknown
TGF-β_2	112	25	1	TGF-R-β_2	542	65	unknown
TGF-β_3	112	25	4				
VEGF-1	121		6	Flt-1	1273	180	13
VEGF-2	165		6	Flk-1/KDR	1367	230	5
VEGF-3	189	45[a]	6				
VEGF-4	205		6	Flt-4	1298	170	5
HGF/SF	728	62 (α) 34 (β)	7	Met	1380	50 (α) 145 (β)	7
PDGF	110–125 (A) 109 (B)	14–18 16	7 22	PDGF-R	1066 (α) 1074 (β)	170 180	4 5

aa, amino acids.
[a] Molecular weight of native VEGF.

The EGF-R (1186 amino acids, 170 kDa) (Table 1), also known as c-erbB, whose gene is located on human chromosome 7, was the first PTK-R to be purified and cloned (Ullrich et al., 1984; Ullrich and Schlessinger, 1990). It is the prototype of a subfamily (subclass-I) of four members, namely erbB-2, erbB-3, erbB-4, and EGF-R itself, and is expressed by most cells. ErbB molecules are characterized by the presence of two extracellular cysteine-rich domains and an intracellular portion with a long C-terminal tail carrying most of the autophosphorylation sites. In addition to the already mentioned transforming growth factor-α and vaccinia growth factor, heregulin (also termed Neu differentiation factor), amphiregulin, cripto, and schwannoma-derived growth factor bind to the EGF-R family. All of these ligands contain a conserved EGF-like domain and are synthesized as transmembrane precursor proteins (Heldin, 1996).

Binding of EGF to its receptor triggers oligomerization and an increase in receptor affinity. The tyrosine kinase domain then autophosphorylates the tyrosine residues of the receptor (Heldin, 1995). Subsequent events include tyrosine phosphorylation of other proteins, including the neu p185 receptor, breakdown of inositol lipids, and increase in calcium concentration (Petch et al., 1990). More specifically, EGF-R stimulation by its ligand leads to Ras activation that in turn activates cytoplasmic protein Ser/Thr protein kinases from both the MEK and Raf families (Zwick et al., 1999). EGF-R signaling is also mediated by STATs, which, following phosphotyrosine-dependent dimerization, bypass classical kinase cascades and enter the nucleus to directly regulate genes (Sadowski et al., 1993; Ruff-Jamison et al., 1995; Xie et al., 1997).

B. EGF and Cancer

It has been noted that components of the EGF-R signaling pathway are over-expressed and/or activated in human breast tumors; moreover, transgenic mouse models have indicated that proto-oncogene *c-myc* and transforming growth factor-α (the latter a member of the EGF family, as mentioned above) strongly synergize to induce mammary tumors (Derynck et al., 1987; Travers et al., 1988). In addition, more recently, Nass and Dickson (1998) examined the ability of *c-myc* expression in cooperation with EGF to abrogate cell cycle regulation in an in vitro mammary epithelial cell model system. These authors report that constitutive, elevated expression of *c-myc* in breast epithelial cells is not sufficient to force the cells through the cell cycle, but rather leads to altered cell cycle progression in response to EGF, with accelerated passage through G_1. The fundamental role of EGF-R, as well as that of its ligands, in the pathogenesis and progression of breast cancer is well recognized. On average, 45% of breast cancers investigated by 40 different groups were shown to be EGF-R positive (Klijn et al., 1992). This positivity was inversely correlated to the presence of estrogen or progesterone receptors and was associated with poor prognosis and higher risk of relapse, although this finding remains controversial. In vivo, between 20 and 60% of EGF-R expressing tumors also express an EGF-R ligand, TGF-α, suggesting that an autocrine loop may be involved in the growth of such tumors (Barrett-Lee et al., 1990; Murray et al., 1993). Finally, EGF-R was recently shown to be associated with an increase in tumorigenesis and to act as a mediator of transformation, evaluated by anchorage-independent growth assay (Ma et al., 1998).

The c-erbB-2/Her-2 (Her-2/*neu*) proto-oncogene encodes a 185-kDa transmembrane tyrosine kinase with a marked degree of homology to the EGF-R. Since the mid-1980s, many publications have shown that Her-2/*neu* is a marker of poor prognosis, shorter overall survival, and biological aggressiveness in breast cancer and in several other epithelial-origin cancers. Her-2/*neu* receptor is commonly overexpressed and involved in autocrine loops in human breast and ovarian cancer (Slamon et al., 1989; Garrett and Workman, 1999). It has also been reported that extracellular ligand-binding domain is shed into the blood of normal individuals and is detected at high levels in women with metastatic breast cancer (Zabrecky et al., 1991). A recent work reported that: 1) c-erbB-2/Her-2 shows a lower sensitivity than CEA and CA 15.3, the most frequently employed tumor markers in breast cancer early diagnosis of relapse and follow-up, in patients with locoregional and metastatic breast tumor; 2) c-erbB-2/Her-2 is an independent prognostic factor in disease-free survival and overall survival; and 3) there is a clear relationship between c-erbB-2/Her-2 overexpression in tissue and in serum (Molina et al., 1998).

Beyond the mechanisms regulating breast cancer cell proliferation, it is necessary to bear in mind that the initial stage of breast cancer development is under hormonal control. In responsive cells, steroid hormones induce a variety of biosynthetic processes leading to increased cell growth. Estrogen-induced stimulation of cellular proliferation involves the binding of the hormone to the estrogen receptor protein that is located predominantly in the cell nucleus. The key to explaining estrogen action lies in defining the mechanisms that come into play between the initial ligand recognition event and the activation of transcription of hormone-responsive genes. On steroid hormone binding, the ligand/receptor complex binds to palindromic DNA binding sites in the vicinity of hormone responsive genes, thereby prompting transcriptional activation. Transcriptional activation by estrogen receptor appears to be mediated by at least two distinct transcription activation factors (TAF), TAF1 and TAF2. Even though TAF1 and TAF2 could potentially act independently, it appears that they interact with the intact receptor in some still undefined way. It has been demonstrated by various biological and/or immunological strategies that condi-

tioned medium from estrogen-treated cells contains numerous proteins, such as protease growth factors and their receptors. These observations prompted interest in understanding the complex "cross talk" occurring between these multiple factors and their regulatory pathways (reviewed in de Cupis and Favoni, 1997).

Several studies have documented transcriptional and/or proliferative synergy between EGF and steroidal hormones (Krusekopf et al., 1991; Modiano et al., 1991) and have shown that progesterone up-regulates EGF-R on the cell membrane (Murphy et al., 1986; Sarup et al., 1988; Modiano et al., 1991). In particular, progestins alone cause a nearly 3-fold increase in the number of EGF-R (Murphy et al., 1986) and a 6-fold increase in EGF mRNA levels (Murphy et al., 1988) in human breast cancer cells. In addition to increasing the number of high affinity EGF-R, progesterone affects the phosphorylation state of the receptor (Modiano et al., 1991). In contrast, progesterone receptors are down-regulated by EGF, thus indicating bi-directional effects of each molecule on the receptor of the other. Moreover, this documented reciprocal regulation suggests the existence of a regulatory loop in which the loss of steroid hormone responsiveness is concurrent with a gain in growth factor-dependent proliferation, as is observed during breast cancer progression (Nicholson et al., 1994; Lupu et al., 1996).

The involvement of EGF-R and its ligand transforming growth factor-α is also recognized in head and neck squamous cell carcinoma. Up-regulation of these molecules, at both the mRNA and protein levels, has been identified as an early event in head and neck carcinogenesis (Rubin Grandis and Tweardy, 1993; Rubin Grandis et al., 1996). In particular, reports show it is likely that increased TGF-α protein expression precedes elevation of EGF-R (Rubin Grandis et al., 1998), indicating that increases of these proteins are chronologically, and perhaps mechanistically, distinct events in the pathogenesis of head and neck squamous cell carcinoma. In addition, EGF-R expression levels in the premalignant lesion appear to be a sensitive predictive factor of the neoplastic potential of dysplastic tissues, thus implying that the receptor protein may serve as a biological marker to identify high-risk subgroups (Rubin Grandis et al., 1998).

In prostate cancer, experimental studies show EGF-R expression to increase with progression, but clinical trials have given rise to contrasting findings. Nevertheless, up-regulation of EGF, TGF-α, and EGF-R in advanced tumor suggests their autocrine expression. In addition, increased EGF levels appear to be associated with the invasive ability of prostate cancer cells (Russell et al., 1998).

Over the past decade, several reports have evinced correlation between the presence of EGF-R and invasive tumors, namely of the prostate, breast, ovary, bladder and lung (Eccles et al., 1995; Ellerbroek et al., 1998), indicating that the receptor plays a role in the malignant phenotype (Damstrup et al., 1998). Moreover, attachment to the basement membrane and cell motility represent two of the first steps involved in tumor cell invasion (Fidler and Nicolson, 1987). Recent findings by Damstrup et al. (1998) indicate that, among a panel of human small cell lung cancer cell lines, only EGF-R positive cell lines, independent of the expression of the enzymes degrading basement membrane (proteinases), had the in vitro invasive phenotype, as demonstrated by the ability to traverse a reconstituted Matrigel membrane.

C. Fibroblast Growth Factors and Receptors

Acidic fibroblast growth factor (FGF) (aFGF; 115 amino acids, 16 KDa) and basic FGF (bFGF; 155 amino acids; 16–18 kDa) (Table 1) belong to the FGF family, which also includes FGF-3 (or *int*-2), FGF-4 (or Kaposi FGF), FGF-5, FGF-6, FGF-7 (or keratinocyte growth factor), FGF-8 (or androgen-induced factor), and FGF-9. These growth factors are modulators of cell proliferation, differentiation, motility, and angiogenesis. Both aFGF and bFGF, whose genes are located on human chromosomes 5 and 4, respectively, have a high affinity for heparin and are found to be associated with extracellular matrix components (Basilico and Moscatelli, 1992).

FGFs bind simultaneously to both low- and high-affinity receptors (Klagsburn and Baird, 1991; Johnson and Williams, 1993). Low-affinity receptors are heparan sulfate proteoglycans. Binding of FGFs to heparin and other glycosaminoglycans protects them from degradation and can retain FGFs in the extracellular matrix as a reservoir. These growth factors bind to cell surface heparan sulfate proteoglycans, but it is not completely clear whether these are functional receptors. Four different genes are currently known to encode distinct high-affinity FGF receptors (Table 1) (K_d ranging from 2×10^{-9} to 5×10^{-10} M): FGF-R1 (flg, 801 amino acids, 160 kDa); FGF-R2 (bek, 800 amino acids, 135 kDa); FGF-R3 (784 amino acids, 135 kDa); and FGF-R4 (778 amino acids, 140 kDa) (Johnson et al., 1991). High-affinity FGF receptors are members of a complex family characterized by the presence of two or three Ig-like domains, a sequence of extracellular acidic residues, and an intracellular PTK domain with a short inserted sequence of 14 amino acids (Fantl et al., 1993). Although their precise ligand-binding specificities remain to be determined, it appears that each receptor in the family binds a subset of members of the FGF ligand family. The genes for FGF-R1 and FGF-R2, located on human chromosome 8 and 10, respectively, may undergo alternative splicing events that give rise to a great variety of products, including soluble receptors (Dionne et al., 1991). Ligand binding to FGF receptors induces dimerization and interchain autophosphorylation. The receptors bind

to and activate PLC-γ and stimulate the phosphoinositol (PI) second messenger pathway (Heldin, 1996).

D. FGFs and Cancer

Numerous studies suggest that alterations in the expression of FGF may contribute to growth deregulation in neoplastic cells (Eguchi et al., 1992; Myoken et al., 1994b). The contribution of aFGF and bFGF to tumor development is supported by the observation that cells transfected with the aFGF or bFGF genes show increased autocrine proliferation in both monolayer cultures and soft agar (Jaye et al., 1988; Sasada et al., 1988). In addition, neutralizing antibodies and antisense oligonucleotides attenuate the growth factor action, indicating that endogenous aFGF and bFGF may contribute to neoplastic cell growth in an autocrine manner (Myoken et al., 1994a). To elucidate the contribution of the autocrine effects of aFGF overexpression to an increased malignant phenotype, Zhang et al. (1998) studied aFGF-transfected MCF-7 breast cancer cells that were retransfected with a vector encoding a truncated FGF-R1. This receptor is truncated immediately after the transmembrane domain (Amaya et al., 1991; Ueno et al., 1992). Although capable of dimerization, it will not cross-phosphorylate a paired wild-type receptor because it lacks the tyrosine kinase domain; this, essentially, nullifies the effect of ligand binding. The "double" transfected MCF-7 cells showed inhibited autocrine growth factor signaling but remained able to produce aFGF, allowing possible paracrine effects to be observed in vivo. Zhang et al. (1998) report that, in ovariectomized mice, truncated receptor expression severely inhibited the ability of aFGF overexpressing breast cancer cells to form tumors in the absence of estrogen. However, rapid formation of large tumors was still observed in estrogen-supplemented mice injected with the same "double"-transfected MCF-7 cells, thus suggesting that paracrine effects of aFGF could act in synergy with mitogenic effects mediated by estrogen. These results imply that aFGF acts as an autocrine modulator of MCF-7 breast cancer cell proliferation under estrogen-depleted conditions. In addition, this growth factor also contributes through paracrine mechanisms of action to the enhancement of tumor growth in estrogen-supplemented animals. Basic FGF, which is a mitogen and a survival factor in fibroblast and endothelial cells, is one of the primary angiogenic factors in breast cancer (Folkman et al., 1989). Paradoxically, as evinced by colony-forming assay (Wang et al., 1998), bFGF also inhibits proliferation of several breast cancer cell lines. Moreover, it has been demonstrated (Wang et al., 1998) that exposure to bFGF promotes drug-induced apoptosis in MCF-7 cells, but has essentially an opposite effect on fibroblasts (NIH3T3), suggesting that bFGF may generate different responses in breast cancer and surrounding cells.

Basic FGF appears to be produced by healthy, normal prostate stromal cells where, acting by an autocrine mechanism of action, it is important for maintaining homeostasis (Story et al., 1989; Sherwood et al., 1992). As prostate cancer occurs and progresses, the production of bFGF becomes androgen-dependent and is regulated by prostate cancer epithelial cells in an autocrine manner (Russell et al., 1998). Because bFGF is angiogenic, as previously mentioned, its increased production in late-stage disease may promote angiogenesis, thus allowing tumor growth and metastases (Folkman, 1990).

In a recent study (Brattstrom et al., 1998), elevated bFGF values were found in sera from patients with nonsmall cell lung cancer, and the authors suggest, in contrast to previous studies carried out on other malignancies, that the bFGF serum levels appear to be a statistically valid prognostic factor.

In ovarian cancer cell lines, bFGF was reported to promote tumor growth and metastases (Di Blasio et al., 1993; Speirs et al., 1993; Crickard et al., 1994), even though no overexpression of bFGF mRNA was revealed in a comparison of malignant and benign tumors (Reynolds et al., 1994). The role of intratumor bFGF as a prognostic marker was recently evaluated in patients with epithelial ovarian cancer (Obermair et al., 1998). It was suggested that cytosolic concentration of bFGF, as well as histological grading and residual tumor mass, affect the overall survival probability. In particular, tumors with high bFGF cytoplasmic levels, which revealed a much greater stromal content, were associated with improved survival. These findings imply that bFGF may induce a fibroblastic response which makes tumors with a high growth factor level less aggressive than those with less stromal tissue (Obermair et al., 1998).

E. Insulin-Like Growth Factors and Receptors

The family of insulin-like growth factor (IGF) ligands includes the single chain polypeptides IGF-I (70 amino acids; 7.6 kDa) and IGF-II (60 amino acids; 7.4 kDa) (Table 1), whose genes are located on human chromosome 12 and 11, respectively (Favoni et al., 1994a). IGFs, which share structural similarity with insulin, play an important role in regulating cell proliferation and differentiation and in suppressing the cell apoptotic pathway (Parrizas and Leroith, 1997). In the fetus, both IGF-I and IGF-II are found at low levels in the serum as well as in most tissues, especially those of mesenchymal origin (Bondy et al., 1990). Synthesized principally by the liver, circulating IGFs are abundant in the newborn human. Although IGF-I primarily mediates the effects of growth hormone after the pituitary axis matures two weeks following birth, the endocrine role of IGF-II is still quite unclear (Voss and Rosenfeld, 1992).

Competitive binding and ligand cross-linking studies were employed to identify the receptors for these growth factors. Although both IGF-I and IGF-II bind weakly to insulin receptor, they have their own specific receptors

on the cell membrane (Rechler and Nissley, 1985; Minniti et al., 1992a). The type-I IGF-receptor (IGF-R) (Table 1), a transmembrane PTK-R, binds both IGF-I and IGF-II with high affinity (K_d 1 and 3 nM, respectively) and is also able to bind insulin, albeit less efficiently (100-fold lower affinity). By contrast, the type-II IGF-R (Table 1), which shows high affinity for IGF-II and significantly lower affinity for IGF-I, is identical with the mannose 6-phosphate-receptor that is involved in the transport of lysosomal enzymes. The type-II IGF-R lacks PTK activity and does not appear to be involved in transducing the IGF-II mitogenic signal (Kiess et al., 1987; Minniti et al., 1992a). Instead, extensive evidence demonstrates that IGF-II exerts its mitogenic effects through the activation of the type-I IGF-R and, in certain cases, through activation of IR (Kiess et al., 1987). It is still uncertain whether and how signaling through the type-II IGF-R occurs. One hypothesis is that by binding to IGF-II, this receptor might regulate the growth factor bioavailability and modulate the growth factor interaction with the type-I IGF-R (Ludwig et al., 1995). Unlike the type-II IGF-R, the type I receptor is structurally related to insulin receptor. Both receptors belong to the type II subgroup of PTK-R (Yarden and Ullrich, 1988). Type-I IGF-R, whose gene is located on human chromosome 15, is a disulfide-linked α_2-β_2 heterodimeric glycoproteinic complex composed of two entirely extracellular α-chains (130 kDa), which provide the growth factor binding site, and two transmembrane-intracellular β-subunits (95 kDa), which possess intrinsic PTK activity (Yarden and Ullrich, 1988; Heldin, 1996). Ligand binding induces autophosphorylation of three closely located tyrosine residues (positions 1131, 1135, and 1136) within the kinase domain, which leads to an increase in the catalytic efficiency of the kinase. Thus, several cellular substrates, such as the insulin receptor substrates 1 and 2 (IRS-1 and IRS-2), the SH2/collagen α-protein and the growth factor-receptor binding protein-10 (GRB-10), are recruited. These substrates then couple the receptor to downstream signaling pathways by serving as binding sites for effector proteins (Rubin and Baserga, 1995). For instance, IRS-1 activates phosphatidyl inositol-3 kinase (PI3K), Ras/MAP cascade (through Grb2/sos), as well as other still poorly identified pathways involving the adapters Nck and Crk (Myers et al., 1994; Beitner-Johnson et al., 1996).

In plasma and in other biological fluids, IGFs are complexed with specific binding proteins (BP). To date, seven proteins have been identified as belonging to the human IGF-BP family; of these, IGF-BP1 (234 amino acids, 25.2 kDa), IGF-BP2 (289 amino acids, 31.3 kDa), IGF-BP3 (264 amino acids, 28.7 kDa), IGF-BP4 (237 amino acids, 25.9 kDa), and IGF-BP5 (252 amino acids, 28.5 kDa) are the most studied and best characterized (Fig. 3). IGF-BP6 (216 amino acids, 22.8 kDa) and IGF-BP7 (251 amino acids, undetermined molecular weight) (Jones and Clemmons, 1995; Swisshelm et al., 1995; Oh et al., 1996) are the most recently identified. All molecular species of IGF-BPs, except IGF-BP7, bind both IGF-I and IGF-II with high affinity and serve to transport IGFs, prolong their half-lives, and modulate their proliferative and anabolic effects on target cells. At the cellular level, IGF-BPs, depending on cell types and IGF-BP species, can either potentiate or inhibit the mitogenic effects of IGFs. The precise molecular mechanisms involved in the interactions among IGF-BPs, IGF-Rs, and their ligands are still unclear. However, IGF-BPs appear to at least regulate the availability of free IGFs for binding with IGF-Rs (Jones and Clemmons, 1995; Kelley et al., 1995). Finally, the recently identified IGF-BP7 (mac25), binds IGFs with lower affinity than do the other molecular species (Oh et al., 1996).

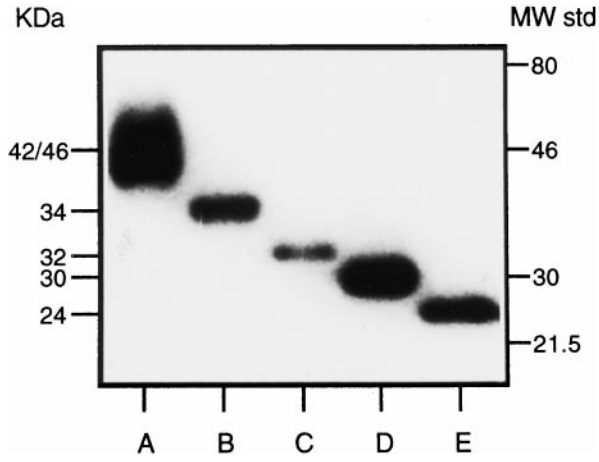


FIG. 3. Pure human recombinant (hr)-IGF-BPs separated by Western Ligand Blot. Molecular weight standard (MW std) is reported on the right axis, while the MW of IGF-BPs is indicated on the left axis. Lines: A = hr-IGF-BP3; B = hr-IGF-BP2; C = hr-IGF-BP5; D = hr-IGF-BP1; E = hr-IGF-BP4.

F. IGF System and Cancer

Studies seeking to elucidate involvement of IGFs in breast tumorigenesis have focused primarily on their potential autocrine/paracrine interactions. Cultured breast cancer epithelial cells express both transmembrane IGF-Rs and are sensitive to the mitogenic stimuli of IGF-I and IGF-II (Rogler et al., 1994). On the other hand, IGF transcripts have rarely been identified in malignant breast epithelial cells either in vitro or in vivo. Rather, IGF transcripts were found prevalently within fibroblasts surrounding the breast epithelium. Thus, extensive evidence suggests that IGFs exert mainly paracrine effects on breast epithelial cells (Gebauer et al., 1998). The finding that malignant breast epithelial cells can also overexpress the type I IGF-R, regardless of IGF source, suggests that enhanced IGF signaling could have physiological relevance in malignancy (Pekonen et al., 1988). Analysis of breast tumor biopsies showed a different pattern of IGF expression. IGF-I mRNA was localized to stromal fibroblasts surrounding normal breast epithelium, whereas high levels

of IGF-II were found in the fibroblasts adjacent to malignant epithelial cells (Yee et al., 1989; Paik, 1992; Rasmussen and Cullen, 1998). Type-I IGF-R is overexpressed in breast tumors as well as in breast cancer cell lines. Resnik et al. (1998) recently reported that type-I IGF-R expression was 14-fold higher in malignant breast tissue than in normal tissue. One possible mechanism underlying this high receptor level is the loss of its repression following mutations of the tumor suppressor gene *p53* (Webster et al., 1996; Werner et al., 1996). In addition, estrogens may also regulate the receptor expression (Stewart et al., 1990; de Cupis et al., 1995). Moreover, receptor autophosphorylation and kinase activity were shown to be two or four times higher in neoplastic than in nondiseased tissues (Resnik et al., 1998). Thus, signaling that occurs through the type-I IGF-R may contribute to the pathogenesis of breast cancer by stimulation of cell proliferation and/or inhibition of apoptosis (Resnicoff et al., 1995). Findings also hint at the possibility that IGF signaling may modulate adhesion and invasion of breast cancer cells at local and distant sites (Scholar and Toews, 1994; Doerr and Jones, 1996; Leventhal and Feldman, 1997; Dunn et al., 1998). The estrogen-induced enhancement of the IGF-mediated mitogenic pathway in estrogen-receptor positive breast epithelium is well documented at both the cellular and molecular levels. In vitro, 17β-estradiol increases the binding of iodinated-IGF-I to its transmembrane receptor, and also increases the expression of the growth factor-receptor itself (de Cupis et al., 1995; de Cupis and Favoni, 1997). In tumors, while there is a positive correlation between type-I IGF-R and estrogen receptor, no correlation has been demonstrated between type-II IGF-R expression and estrogen receptor status (Pekonen et al., 1988; Mathieu et al., 1990).

Breast cancer cells also secrete various molecular species of IGF-BPs. The predominant secreted IGF-BP appears to correlate with estrogen receptor status of the cell. Estrogen-receptor-negative cells secrete IGF-BP3 and IGF-BP4 as major species, and IGF-BP6 as a minor protein; by contrast estrogen-receptor-positive cells synthesize IGF-BP2 and IGF-BP4 as major species and IGF-BP3 and IGF-BP5 as minor species (Clemmons et al., 1990). These different patterns of IGF-BP expression imply that the IGF system in breast cancer is complex and that the biological significance of cellular response to IGF-BPs may differ according to estrogen-responsiveness. In addition, recent studies have indicated that IGF-BPs, especially IGF-BP3, strongly inhibit breast cancer cell proliferation in an IGF-independent manner. The IGF-independent action of IGF-BP3 requires interaction with cell surface proteins, most likely putative IGF-BP3-specific receptors (Oh, 1998). Recent reports suggest that IGF-BP3 appears to be a major factor in a negative control system involved in regulating breast cancer cell proliferation in vitro (Oh et al., 1995; Gucev et al., 1996). Several studies have been conducted to evaluate whether IGF family members could be prognostic indicators of breast cancers. Although in vitro data suggest that IGF expression should be a reliable prognostic marker in breast tumors, the limited clinical evidence obtained to date does not support these findings (Barni et al., 1994; Bhatavdekar et al., 1994). Similarly to what was ascertained for estrogen receptor, the type-I IGF-R has also been identified as a potentially valuable prognostic marker (Lee et al., 1998). Finally, when analyzed as prognostic factors, high levels of IGF-BPs are generally poor, although the physiological mechanism underlying this observation is not known (Yee et al., 1994; Lee et al., 1998).

In healthy prostate, IGF-I and IGF-II are produced by stromal cells, whereas normal epithelial cells express the type-I IGF-R. Both epithelial and stromal cells secrete mainly IGF-BP4, together with IGF-BP2 and IGF-BP3. On the other hand, controversy surrounds the IGF-mitogenic loop in prostate cancer. Kimura et al. (1996) showed that the DU-145 prostate cancer cell line could proliferate in response to IGF-I, but that it does not produce this protein, suggesting a paracrine mode of action of this growth factor. However, other authors describe an autocrine loop for IGF-I in three different prostate cancer cell lines, which also display constitutively autophosphorylated type-I IGF-R (Pietrzowski et al., 1993). The balance of IGF-BPs produced by cancer cells varies, depending on the cell lines examined. There is also evidence that some IGF-BPs may be under androgen regulation (Russell et al., 1998). Particularly, in vitro data appear to suggest that androgen may indirectly modulate IGF-induced proliferation of prostate cancer cells by regulating IGF-BP3 production (Marcelli et al., 1995). Furthermore, in advanced prostate cancer, IGFs appear to be involved in the development of bone metastasis (Chevalley et al., 1996).

In colorectal cancer, the IGF-II gene was reported to be overexpressed in 30 to 40% of the tumors tested (Lambert et al., 1990, 1991). Recently, it has been suggested that IGF-II modulates, by a paracrine mechanism of action, cellular proliferation of human colorectal cancer cell lines through binding to the type-I IGF-R (Lahm et al., 1994; Lamonerie et al., 1995). Kawamoto et al. (1998) indicated that IGF-II expression, evaluated by immunohistochemical staining of tissue samples from 92 colorectal cancer patients, was correlated with tumor progression, clinic-pathological factors, and patient survival. These findings led the authors to conclude that IGF-II staining might be a useful prognostic factor in colorectal cancer. Finally, a recent in vitro study (Akagi et al., 1998) performed on human colon cancer cell lines demonstrated that IGF-I is able to increase the expression of vascular endothelial growth factor (VEGF, see below), a potent and unique angiogenic protein.

The IGF system is also involved in the modulation of both small cell lung cancer (SCLC) and nonsmall cell lung cancer (N-SCLC) proliferation. Primary lung tu-

mors possess IGF-I binding sites as detected by autoradiography and/or monoclonal antibodies (Maculay, 1992; Kaiser et al., 1993). In addition, iodinated ligand-binding assays revealed two classes of IGF-R (high- and low-affinity) on SCLC cell lines (Rotsch et al., 1992). In a previous study, we demonstrated the presence of one class of high-affinity functional type-I IGF-R on a pool of human N-SCLC cell lines belonging to adenocarcinoma and squamous carcinoma histological subtypes (Favoni et al., 1994a). Data on the type-II IGF-R in human lung cancer are scarce. However, expression of this receptor has been demonstrated in both SCLC and N-SCLC cells. Schardt et al. (1993) characterized type-II IGF-R in SCLC cell lines and demonstrated a 10- to 15-fold higher affinity of the IGF-II ligand for the type-I than for the type-II receptor. Immunoreactive IGF-I is detectable in primary lung tumor tissues to a greater extent than in normal lung. Furthermore, the mitogenic peptide is also detectable both in extracted SCLC and N-SCLC cells and in their conditioned media (Maculay, 1992; Favoni et al., 1994a). It has, however, been demonstrated that IGF-I levels in lung cancer patients' sera are not related to the bulk of disease or response to treatment. Beyond expressing the growth factor and cell surface receptor, lung cancer cells can also synthesize and secrete IGF-BPs, even if the pattern of their expression differs qualitatively and quantitatively depending on the model analyzed (Kiefer et al., 1991; Maculay, 1992; Favoni et al., 1994a). In agreement with the proposal of Sporn and Todaro (1980), the production of the mitogenic growth factor, the expression of the specific receptor and the carrier proteins, together with cellular sensitivity to growth factor action, are conditions that should substantiate the hypothesis of an autocrine mechanism of action in lung cancer cells. Nevertheless, available experimental data do not definitively exclude a paracrine role for the IGF system in lung cancer (Jaques et al., 1988; Maculay, 1992; Rotsch et al., 1992; Favoni et al., 1994a).

G. Transforming Growth Factors and Receptors

Transforming growth factors (TGFs) (Table 1) are polypeptides that, when originally isolated from viral-transformed rodent cells, were found to convert some normal cells to a transformed phenotype. Thus, in the presence of these growth factors, cultured fibroblasts pile up and decrease their anchorage-dependence, but do not become neoplastic (Folkman and Klagsbrun, 1987). Furthermore, it has been demonstrated that TGFs are also angiogenic in vivo. Two structurally distinct TGFs, TGF-α and TGF-β, have been purified and their structures determined by protein sequencing and cDNA cloning. TGF-α, whose gene is located on human chromosome 2, is a small integral membrane protein (50 amino acids; 6 kDa) which shares biological and structural properties with EGF (35% homology) and exerts its action through EGF-R (Yarden and Ullrich, 1988; Massagué et al., 1994; Heldin, 1996). TGF-β is a pleiotropic growth factor involved in tissue remodelling, wound repair, development, and hematopoiesis, but its predominant action is to inhibit cell growth. There are three structurally-related TGF-β isoforms, TGF-β1, TGF-β2, and TGF-β3, which are encoded by different genes on human chromosomes 19, 1, and 14, respectively. The expressed proteins (25 kDa) are biologically inactive disulfide-linked dimers that are cleaved to active dimers of 112-amino acid disulfide-linked peptides (Miller et al., 1990). These molecules have been shown to exert their actions by binding to heteromeric complexes of serine/threonine kinase receptors (Massagué et al., 1994). Both type I and type II TGF-β receptors (Table 1) have small cysteine-rich domains; the type I receptors have a characteristic region rich in glycine-serine residues domain in their cytoplasmic juxtamembrane domain. Both receptors are needed for signaling, and the cytoplasmic parts of the receptors are not interchangeable (Heldin, 1995). TGF-β binding induces formation of a hetero-oligomeric complex of the type I and type II receptors, most likely a heterotetramer containing two receptors of each type (Yamashita et al., 1994a). In particular, the type II receptor, which also exists as a dimer in the absence of ligand and has a constitutively activate kinase, is the first to bind the growth factor. The resulting complex then recruits the type I receptor, which cannot bind TGF-β without the type II receptor. Thus, phosphorylation of the type I receptor on serine residues occurs in the glycine-serine domain (Chen and Derynck, 1994; Henis et al., 1994). The phosphorylation presumably activates the type I receptor kinase, which acts on downstream components in the signal transduction pathway (Heldin, 1995). In addition to the high-affinity type-I and type II receptors, TGF-β also binds to the low-affinity type III receptors, which include β-glycan and the endothelial cell-specific type III receptor CD105 (Cheifetz et al., 1992). Beta-glycan has not been shown to transduce signals, but it may function to concentrate TGF-β on the cell surface and to present the ligand to its other receptors. It appears that coexpression of type III and type II receptors increases the ability of the high-affinity receptor to bind the ligand (Lopez-Casillas et al., 1994). Little is known about the possible role of the specialized vascular endothelial cell receptor CD105 in the TGF-β signaling pathways, although it has been demonstrated to form heteromeric complexes with the signaling receptors on endothelial cells (Yamashita et al., 1994a).

H. TGFs and Cancer

TGF-β ligands and receptors are expressed by normal breast epithelial cells. In vivo, TGF-β appears to regulate the normal development of ductal and lobulary epithelium in the mammary gland (Jhappan et al., 1993). Moreover, in the adult, this growth factor seems to mediate the cell death and restructuring processes that take place during postlactation involution (Strange et

al., 1992). Beyond these physiological functions, there is considerable evidence that TGF-β is pivotally involved in breast cancer (Reiss and Barcellos-Hoff, 1997). Several published findings indicate that endogenous TGF-βs expressed by tumor cells are autocrine modulators of cell proliferation (Norgaard et al., 1995; Gold, 1999). In addition, estradiol-induced cell proliferation in hormone-dependent breast cancer cell lines is known to be associated with a decrease in TGF-β2 and TGF-β3 mRNA levels (Arrick et al., 1990). Furthermore, loss of autocrine growth factor-mediated growth regulation due to down-regulation or mutation of TGF-β receptors has been associated with tumor progression (Brattain et al., 1996; Koli and Arteaga, 1996). Recently, a particular somatic missense mutation in the TGF-β receptor was identified (Chen et al., 1998); it results in a serine to tyrosine substitution at codon 387, within the catalytic core of TGF-β-R1 serine-threonine kinase. This mutation disrupts the signaling function of the receptor. It has been proposed that the inactivation of the TGF-β signaling pathway is probably a relatively late event because the mutation was found predominantly in metastatic lesions (Chen et al., 1998). However, the growth inhibitory effect of TGF-β on breast cancer cell line proliferation, documented by in vitro studies, was not confirmed in experimental animal models. Several reports suggest a positive association between TGF-βs and breast cancer progression, owing to enhanced angiogenesis and suppressed host immune surveillance (Arteaga et al., 1996). A recent study (Li et al., 1998) carried out on 80 patients with early stage breast cancer proposed that the plasma levels of TGF-β3 and type III receptor CD105-TGF-β3 complex may be of prognostic value in the early detection of breast cancer metastasis.

In nondiseased prostate, TGF-βs exert a role in growth regulation by counterbalancing the mitogenic effects of various growth factors (Russell et al., 1998). Conversely, increasing intracellular expression of TGF-β1 mRNA and protein, detected in both epithelial and stromal cells, seems to be important in prostate cancer progression, even though its exact involvement remains uncertain (Truong et al., 1993). In addition, it has been suggested that the progression of prostate cancer is associated with TGF-β1 switching from an autocrine/paracrine to a juxtacrine mechanism of action (Russell et al., 1998). Studies on human prostatic cancer cell lines suggest that changes in sensitivity to TGF-β1 may be related to progression of the disease. Kim et al. (1996) documented the ability of TGF-β1 to inhibit the proliferation of androgen-independent cell lines but not the growth of androgen-sensitive LNCaP cell lines. The insensitivity of LNCaP cells to TGF-β has been attributed to a genetic change in their TGF-β receptor I gene. This could provide a possible mechanistic explanation for the ability of the prostate cancer cell to escape the growth-inhibitory effects of TGF-β. Furthermore, it has been shown that TGF-β can modulate extracellular matrix metalloprotease production and can stimulate adhesion of prostate cancer cells to bone cells. These observations imply the potential involvement of TGF-β in the promotion of prostate cancer metastases (Sehgal et al., 1996). Finally, in view of the role of TGF-β in angiogenesis and as an immunoregulatory molecule, TGF-β secretion could have important effects on prostate cancer cell environment.

TGF-β1 arrests the cell cycle by transcriptionally activating a series of cyclin-dependent kinase inhibitors including $p21^{waf1/cip1}$ (Datto et al., 1995; Halevy et al., 1995). Because both $p21^{waf1/cip1}$ and TGF-β1 modulate apoptosis and cell cycle progression, protein levels should be correlated with biological outcomes, e.g., survival. It has recently been reported that concordant expression of TGF-β1 and $p21^{waf1/cip1}$ levels (i.e., high and high, or low and low, protein expression) detected by immunohistochemical analysis (Bennett et al., 1998) predicted 70% disease-free survival at 2000 days follow-up in N-SCLC patients. Although currently available models do not clearly explain these findings, it appears that analysis of both TGF-β1 and $p21^{waf1/cip1}$ may provide useful information concerning the survival of these patients.

I. Vascular Endothelial Growth Factor and Receptors

The dimeric molecule VEGF, also known as vascular permeability factor, was first purified from media conditioned by bovine pituitary folliculostellate cells. VEGF owes its discovery to its ability to stimulate angiogenesis by increasing vascular permeability (Senger et al., 1983) and by acting as an endothelial cell mitogen (Ferrara and Henzel, 1989). Four different isoforms of VEGF transcripts (Table 1) encoding polypeptides of 206, 189, 165, and 121 amino acids have been reported to be expressed in human cells; each of these isoforms possesses different biological activity (Houck et al., 1992). VEGF121 and VEGF165 are secreted in soluble forms, whereas the two larger isoforms, VEGF189 and VEGF206, remain associated with cells because of their stronger affinities for cell-surface proteoglycans. The smallest isoform does not bind heparin, whereas the inclusion of more cationic exons in VEGF165 and VEGF189 confers heparin-binding properties (Scott et al., 1998). The largest isoform, VEGF206, has been identified only in a fetal liver library, and little is known about its biological relevance (Houck et al., 1991).

The family of VEGF receptors (Table 1) contains three members, Flt, Flk-1/KDR and Flt-4, each characterized by the presence of seven extracellular Ig-like domains and an intracellular PTK domain. These receptors are expressed predominantly on endothelial cells and have been shown to be of importance in mediating angiogenic response (Heldin, 1996).

J. VEGF and Cancer

VEGF expression has been detected at the mRNA and protein level in a number of malignancies (Brown et al., 1993; Sato et al., 1994; Guidi et al., 1995; Mattern et al., 1995; Olson et al., 1995; Maeda et al., 1996; Mise et al., 1996). In human breast carcinoma, VEGF mRNA expression, as detected by in situ hybridization, was reported to be higher in neoplastic cells than in normal ductal cells (Brown et al., 1995). In addition, the levels of growth factor protein were found to correlate with increased microvessel density and early relapse in the same disease (Toi et al., 1994). Recent studies have shown that VEGF mRNA and protein levels are significantly higher in tumor samples than in matched normal tissues (Yoshiji et al., 1996; Scott et al., 1998). Furthermore, Scott et al. (1998) demonstrated that VEGF mRNA level was elevated in breast tumors expressing the EGF-R, while no differences according to estrogen receptor status or nodal status were observed. Recently, a posttranscriptional mechanism modulating VEGF expression has been identified. The mRNA of growth factors such as VEGF is characterized by a long 5′untranslated region with complex secondary structures that render them inefficiently translated (Kevil et al., 1996). The polypeptide eIF-4E unwinds the 5′untranslated region of the target mRNA, thus facilitating the identification of the translation start site by ribosomes. It has been shown that elevated levels of eIF-4E are associated with increased levels of VEGF protein and increased growth rate (Kevil et al., 1996).

VEGF overexpression was also observed in the sera of N-SCLC patients (Brattstrom et al., 1998; Takigawa et al., 1998), and was related to an increase in microvessel density (Mattern et al., 1995, 1996). However, contrary to the observation reported for breast cancer, the level of VEGF in lung cancer patients appeared to be unrelated to tumor burden, thus casting doubt on the usefulness of this angiogenic growth factor as a clinically reliable tumor marker (Brattstrom et al., 1998; Takigawa et al., 1998). In human lung cancer, increased VEGF level was found to be correlated with nuclear accumulation of p53 (Mattern et al., 1995; Fontanini et al., 1997). The expression of this angiogenic growth factor is induced by hypoxia (Forsythe et al., 1996), a feature common to all solid tumors. Studies have shown that hypoxia increases VEGF expression within 3 to 6 h, whereas normalization of oxygen tension causes cellular VEGF mRNA to return to baseline levels (Ikeda et al., 1995; Shima et al., 1995). Hypoxic induction of VEGF may be modulated by an increase in transcription and/or stabilization of its mRNA (Levy et al., 1995, 1996). Furthermore, a mutationally activated *ras* oncogene (Larcher et al., 1996; Mazue et al., 1996) or p53 (Kieser et al., 1994) can act synergistically with hypoxia to induce VEGF expression. In contrast, wild-type p53 down-regulates VEGF promoter activity (Mukhopadhyay et al., 1995) and up-regulates the expression of the antiangiogenic factor thrombospondin-1 (Dameron et al., 1994). However, a recent report (Ambs et al., 1998) found no evidence of a direct regulation of VEGF by p53 in N-SCLC tissues.

Data from several laboratories indicate that microvessel counts are strong prognostic factors in human colorectal cancer (Takahashi et al., 1995, 1996, 1998). The finding that VEGF expression correlates with microvessel counts has implicated this growth factor in the regulation of colon cancer angiogenesis (Takahashi et al., 1995, 1996, 1998). Furthermore, on a pool of colon cancer specimens, an association of mutant p53 expression with VEGF and vessel count was observed (Takahashi et al., 1998); this finding appears to suggest that poor prognosis associated with p53 mutation (Kastrinakis et al., 1995) may be due, at least in part, to the ability of p53 protein to promote angiogenesis (Takahashi et al., 1998). Tokunaga et al. (1998) recently reported that, together with the detection of VEGF level, it is also important to examine the growth factor isoform patterns in order to predict the prognosis of colon cancer patients. In addition to hypoxia, factors such as EGF, bFGF, TGF-α, TGF-β, IL-6, and other cytokines are now being characterized as mediators of VEGF expression in both neoplastic and normal cells. An experimental study by Akagi et al. (1998) showed that IGF-I, but not IGF-BPs, induces VEGF mRNA and protein expression in colon carcinoma cell lines. The increased growth factor level appears to be due to an increase in gene transcription without significant alteration of the mRNA half-life.

Ovarian cancer is characterized by widespread i.p. carcinomatosis and formation of large volumes of ascitic fluid. VEGF may play a major role in the progression of ovarian cancer by modulating tumor proliferation through its promotion of tumor angiogenesis. Moreover, the growth factor may be involved in ascites production by stimulating vascular permeability (Mesiano et al., 1998). Although VEGF has been detected in ovarian cancer (Boocock et al., 1995; Brown et al., 1995; Abu-Jawdeh et al., 1996; Paley et al., 1997), its role as a regulator of angiogenesis is not completely elucidated. However, microvessel density and the level of VEGF expression directly correlate with poor prognosis in ovarian cancer, thus suggesting that angiogenesis, mediated at least in part by VEGF, influences disease progression (Boocock et al., 1995; Abu-Jawdeh et al., 1996; Paley et al., 1997). Finally, Tempfer et al. (1998) recently reported that VEGF appears to be an additional factor for predicting the clinical outcome of epithelial ovarian cancer patients.

K. Hepatocyte Growth Factor/Scatter Factor and Receptor

Hepatocyte growth factor (HGF) (Table 1), first identified as a mitogen for hepatocytes (Nakamura et al., 1984), has also been described as a growth modulator of kidney cells, melanocytes, keratinocytes, and other cell

lines in vitro (Igawa et al., 1991; Kan et al., 1991; Matsumoto et al., 1991). Scatter factor (SF), originally described as a cytokine dispersing cohesive epithelial colonies and stimulating cell motility, was subsequently shown to be identical with HGF (Stoker et al., 1987; Weidner et al., 1990, 1991; Naldini et al., 1991b). Early studies revealed that HGF/SF is produced by cells of mesenchymal origin, such as fibroblasts, and acts on epithelial cells (Sonnenberg et al., 1993). Recently, it was reported that cells of epithelial origin also express HGF/SF (Olivero et al., 1996; Tuck et al., 1996; Jin et al., 1997). In vitro studies showed HGF/SF to be a morphogenetic (Montesano et al., 1991) and an angiogenic factor (Bussolino et al., 1992). Experimental studies indicated that HGF/SF might also induce endothelial secretion of plasminogen activators that are required during the early stages of angiogenesis, in which endothelial cells degrade the extracellular matrix (Grant et al., 1993). The growth factor is produced in an immature form and is then processed by a proteolytic cleavage into a glycosylated heterodimer of a heavy α-chain (62 kDa) and a light β-chain (34–32 kDa). The α-chain contains four kringle domains, the first two of which are necessary for scatter activity; the complete protein, on the other hand, appears to be involved in mitogenic activity. The β-chain shows strong homology with the catalytic domain of serine proteases, but does not possess enzymatic activity because the catalytic site is different (Matsumoto and Nakamura, 1992; Bellusci et al., 1994).

The HGF/SF receptor was first identified as the product of the *c-met* proto-oncogene (Naldini et al., 1991a) (Table 1). The mature protein is a heterodimer composed of 50-kDa α- and 145-kDa β-subunits. The β-chain extends over the membrane and contains the catalytic domain, whereas the α-chain remains extracellular. The receptor is autophosphorylated inside the kinase domain, thereby increasing its catalytic activity, as well in the C-terminal tail where two closely related tyrosine residues account for binding of a number of different signal transduction molecules (Bellusci et al., 1994; Heldin, 1996). Furthermore, several isoforms produced by either alternative splicing or posttranscriptional modifications have been characterized (Prat et al., 1991; Rodrigues et al., 1991).

L. HGF/SF and Cancer

Cell scatter activity of HGF/SF was identified in several types of tumor cells expressing the HGF/SF receptor (Nakamura, 1991). In endothelium, the growth factor was demonstrated to induce chemotactic activity and cell proliferation both in vitro and in vivo (Bussolino et al., 1992; Grant et al., 1993). These observations indicate that up-regulation of the production and/or activation of HGF may promote not only tumor spreading, but also neovascularization.

In human breast cancer, enzymatic immunoassay showed that HGF/SF protein concentration is significantly higher in tumor tissues than in adjacent normal tissues (Yamashita et al., 1994b). On the other hand, immunocytochemical analysis revealed that only stromal cells, not tumor cells or ductal epithelial cells, express c-met in breast cancer tissues. In addition, HGF/SF expression was identified in only a few cultured breast cancer cell lines in vitro, thus indicating that the growth factor is involved as a paracrine modulator in breast cancer tissues (Seslar et al., 1993; Tuck et al., 1996). HGF/SF serum level has often been found to be elevated in breast cancer patients, particularly in those with distant metastases (Taniguchi et al., 1995). Moreover, the increase of circulating HGF/SF was significantly correlated with tumor size, nodal metastasis, and histological evidence of venous invasion, which are conventional prognostic markers (Roses et al., 1982; Rosen, 1983). Toi et al. (1998) recently proposed the increase in serum levels of the growth factor as a novel, significant, and independent indicator of poor prognosis in primary breast cancer, particularly in node positive patients. Indeed, 60% of patients with distant metastases showed an increase in serum HGF/SF level. Thus, the same authors suggested that up-regulation of the circulating HGF is strongly associated with systemic tumor spread and with relapse in primary breast cancer patients (Toi et al., 1998). In keeping with these findings, Wu et al. (1998) reported that increased serum HGF/SF levels in gastric cancer patients were associated with disease progression. Moreover, these authors demonstrated that patients with lower serum growth factor values live longer than patients with higher serum HGF. Nakamura et al. (1997) observed that HGF/SF promotes mitogenesis, motogenesis, and invasion of human carcinoma cell lines, including two lung cancer lines. The authors demonstrated that these cell lines secrete factors, such as bFGF and platelet-derived growth factor that promote HGF/SF production by stromal fibroblasts. These findings indicate that there is a mutual interaction between carcinoma cells and stromal fibroblasts that promotes the migration and invasion of carcinoma cells. It is noteworthy that proteolytic degradation of the extracellular matrix, disruption of cell adhesion, and increased cell motility are key elements of tumor cell invasion. HGF/SF induces tyrosine phosphorylation of β-catenin, which in turn contributes to the promotion of cell motility by disrupting epithelial tumor cell-cell adhesion, as demonstrated in an epithelial colorectal cell line (Hiscox and Jiang, 1999). In addition, HGF/SF induces the expression of the urokinase plasminogen activator and its receptor (Pepper et al., 1992), which then activate the proteinase cascade that promotes degradation of the extracellular matrix.

M. Platelet-Derived Growth Factor and Receptor

Platelet-derived growth factor (PDGF) (Table 1) is mitogenic for connective tissue cells and glial cells and is involved in wound healing. It is also a chemoattractant

for fibroblasts, smooth muscle cells, neutrophils, and monocytes (Ross et al., 1986). Although PDGF synthesis and secretion were first observed in platelets, other cell types (e.g., monocytes/macrophages, megakaryocytes, vascular smooth muscle cells, and embryonic cells) have been found to produce this growth factor (Antoniades and Owen, 1988; Ogasawara and Subuska, 1988). Under physiological conditions, the in vivo mitogenic activity of the PDGF is confined to sites of injury and repair; normal plasma does not contain detectable levels of growth factor. Secreted PDGF is quickly bound to circulating binding proteins such as β_2-macroglobulin (Bowen-Pope et al., 1984; Raines et al., 1984), and such binding inhibits the interaction of PDGF with its receptor. Functional PDGF is secreted as a dimer of disulfide-linked A and B chains: PDGF-AA, PDGF-BB, or PDGF-AB. All three isoforms are produced naturally. The mature A and B chains have 60% homology with eight conserved cysteinic residues in each chain. The A chain, whose gene is located on human chromosome 7, occurs in two different variants (110/125 amino acids; 14–18 kDa) arising from alternative splicing in which the three C-terminal amino acids in the short form are replaced by 18 different amino acids in the long form. The gene coding for the human PDGF B chain (109 amino acids; 16 kDa), located on human chromosome 22, is the *sis* proto-oncogene.

PDGF receptors α (1066 amino acids; 170 kDa) and β (1074 amino acids; 180 kDa) (Table 1) are single transmembrane glycoproteins with five extracellular Ig-like domains and an intracellular tyrosine kinase domain split by an inserted sequence of 100 amino acids. Genes for α- and β-PDGF receptor (PDGF-R) are located on human chromosomes 4 and 5, respectively. These receptors, belonging to the subclass III of RTK, are structurally related to colony-stimulating factor 1 receptor (also known as the proto-oncogene product fms), stem cell factor receptor (Kit), and Flt3/Flk2 (Heldin, 1996). Binding of divalent PDGF (AA, AB, or BB) leads to receptor dimerization with three possible configurations ($\alpha\alpha$, $\alpha\beta$, or $\beta\beta$). The PDGF-R α-subunit binds both PDGF A and B chains, whereas the β-subunit binds only PDGF B chains. This specificity infers that PDGF-AA binds only to PDGF-R $\alpha\alpha$ dimers, PDGF-AB binds to R $\alpha\alpha$ and $\alpha\beta$ dimers, and PDGF-BB binds to all three possible receptor configurations (Heldin, 1995). The kinase insert domains contain several autophosphorylation sites and thereby function to mediate interactions with several SH2 domain-containing signal transduction molecules; moreover, autophosphorylation sites are found also in the juxtamembrane regions, in the C-terminal tails, and inside the kinase domains (Heldin, 1996). There are certain differences in the signals transduced via $\alpha\alpha$ and $\beta\beta$ receptor homodimers, for instance with regard to chemotaxis stimulation. Furthermore, PDGF-AB, which preferentially induces $\alpha\beta$ receptor dimers, leads to a stronger mitogenic response than other PDGF isoforms. This receptor property could be related to the presence of unique autophosphorylation sites found only in the $\alpha\beta$ heterodimer receptor and not seen in the homodimeric receptor, which may mediate interaction with additional signal transduction molecules. Thus, the response to PDGF depends on both the particular isoform of the growth factor and the number of α and β receptors expressed on the target cells (Heldin, 1995).

N. PDGF and Cancer

As previously mentioned, the PDGF B chain shares 90% homology with the transforming protein of the simian sarcoma virus (P28v-sis), which is able to induce tumors and to transform cells expressing PDGF-R. This knowledge underlies the belief that PDGF may be involved in the process of retrovirus-induced neoplasia (Waterfield et al., 1983). In addition, the cellular C-sis homolog has been pointed out as playing a similar role in nonvirally induced tumors. However, the precise involvement of this growth factor in malignancy has been quite difficult to define. PDGF is a ubiquitous mitogenic peptide found not only in a wide range of tumors, but also in stromal and mesenchymal cells, thus suggesting a complex regulatory network.

In vitro studies have indicated that, whereas PDGF is secreted by a number of breast cancer cell lines, PDGF-Rs have not been identified in these models. These findings imply that any effect ensuing from PDGF synthesis and secretion may be paracrine rather than autocrine (Bronzert et al., 1987; Lippman et al., 1987). Conversely, other workers detected intracellular PDGF-Rs in cell lines previously thought to be PDGF-R negative (Rakowicz-Szulczynska and Koprowski, 1991). In addition, a mitogenic activity of this growth factor on breast cancer cell lines and the presence of the PDGF receptor are documented (Ginsburg and Vonderhaar, 1991). These observations have prompted the suggestion that previous investigators may have detected the mitogenic effects of PDGF when using noncharcoal stripped serum (adult or fetal bovine) containing high levels of endogenous PDGF. Ariad et al. (1991), measuring PDGF plasma levels in breast cancer patients, demonstrated that elevated levels of circulating growth factor correlate both with greater bulk of disease and with poorer prognosis in these women. A subsequent study was designed to examine tissue expression of various PDGF isoforms in patients with breast cancer in an attempt to elucidate the involvement of PDGF in the biological control of this tumor. The authors demonstrated a lower survival in patients with advanced breast cancer who were positive for PDGF by using tissue immunostaining (Seymour et al., 1993; Seymour and Bezwoda, 1994). The site of action of PDGF in clinical breast cancer is not completely clear. It is possible that PDGF produced by breast tumors not only acts in an autocrine loop but also exerts paracrine effects on stromal cells, including PDGF-R-

expressing fibroblasts surrounding breast cancer epithelial cells (Seymour and Bezwoda, 1994).

The expression of PDGF genes in human lung cancer cell lines has been described only in *N*-SCL carcinoma (Soderdahl et al., 1988). Moreover, PDGF-AA and PDGF-BB were reported to be localized in airway epithelial cells and in mesenchymal cells in the embryonic and fetal rat lung by immunohistochemistry and Western blotting (Han et al., 1992). The finding that immunoreactive expression is increased in the late pseudoglandular stage and in the canalicular stage is intriguing, because both fetal tissue and tumor cells in the lung are immature. A recent study (Kawai et al., 1997) demonstrated that positive PDGF B chain staining is associated with poor prognosis in patients with lung carcinoma, irrespective of age, sex, stage, and degree of cell differentiation.

The effect of PDGF and expression of PDGF-R were recently examined in neoplastic and nonneoplastic ovarian epithelial cells (Dabrow et al., 1998). The magnitude of the mitogenic PDGF activity appears to be related to the passage number in culture, because growth stimulation was maximized in those cells cultured the longest in vitro. The authors suggest that ovarian epithelial cells in later passages may have accumulated genetic alterations, leading to an increased sensitivity to growth factor (Dabrow et al., 1998). The importance of the PDGF system in ovarian cancer may be reflected in the longer median survival of patients who retain the PDGF-R β versus those who do not. Although this retrospective study by Dabrow and colleagues (Dabrow et al., 1998) was performed on a small number of patients, the median relapse-free survival of patients positive for PDGF-R β staining was significantly prolonged. The loss of PDGF-Rs in patients with a short median survival may be indicative of independence of growth hormonal influences to cellular proliferation.

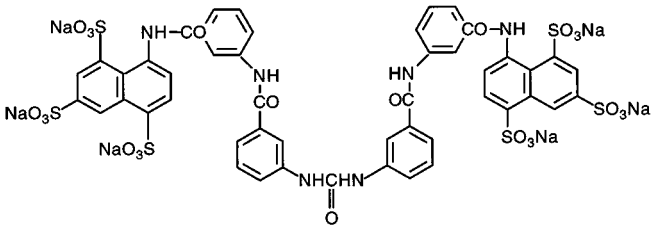
III. Pharmacological Interference with Growth Factor Signaling Pathways

Growth factor signaling pathways, which are subverted in cancer cells, provide potential targets for therapeutic intervention. For this reason, molecules have been and continue to be developed with the aim of interfering with these crucial processes. In the following section, data from preclinical and clinical studies concerning signaling inhibitor compounds are reviewed and discussed.

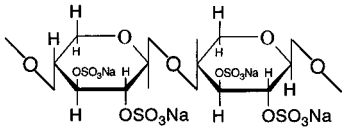
A. Growth Factor Neutralization

Counteraction of growth factors represents one of the potential intervention targets in the signaling process. Nonselective inhibition of growth factor activity can be achieved by using molecules such as suramin or pentosan polysulfates. These agents are able to bind mitogenic peptides and thereby neutralize their growth-promoting action.

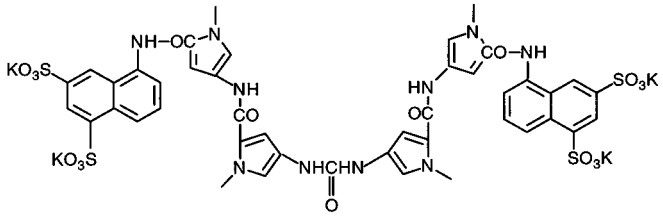
Suramin (Fig. 4) is a polysulfonated naphthylurea that was used during World War I for the treatment of trypanosomiasis and onchocerciasis (Webster, 1985). Investigators have since identified a wide range of biological properties for this compound that appear to be related, at least in part, to its structural characteristics. The presence of three sulfonic acid groups coupled to naphthalene rings on each side of the molecule (Fig. 4) makes suramin a highly charged polyanionic compound, similar to other naturally occurring polymers (Gallagher et al., 1986). Among the most important functions of this drug is its antitumor activity, owing primarily to its interference with growth factor binding and the subsequent loss of the mitogenic signal. In particular, several reports concerning both preclinical and clinical studies clearly demonstrate that suramin inhibits interaction of EGF, bFGF, IGFs, TGFα, and PDGF with their receptors (Pollak and Richard, 1990; Minniti et al., 1992b; Wade et al., 1992; Ravera et al., 1993; Favoni et al., 1994b). The mechanism by which the drug interferes with the growth factor signaling pathways is believed to



suramin



Na-pentosan polysulfate



PNU145156E

FIG. 4. Inhibition of growth factor activity can be achieved by using molecules such as the polysulfonated naphthylurea suramin, its less toxic analog pentosan polysulfate, and the distamycin A derivative PNU145156E, which are able to bind mitogenic peptides, thus neutralizing their growth-promoting action.

lie in its ability to capture the mitogenic peptide, thereby reducing the amount of growth factor available. Unfortunately, the clinical use of this compound is limited by its excessive toxicity and unpredictable pharmacokinetic action. Furthermore, higher concentrations of drugs are required to obtain in vivo the same inhibitory effect observed in vitro because suramin is highly serum-protein bound, mainly to albumin (Lopez-Lopez et al., 1992). For this reason, a number of studies attempted to optimize the administration of suramin in order to maintain a therapeutic dose without excessive peak levels (Scher et al., 1992; Eisenberger and Reyno, 1994). Furthermore, in the search for more efficacious and less toxic drugs, Zugmaier et al. (1992) demonstrated that the suramin analog heparinoid pentosan polysulfate (PPS) (Fig. 4), as well as other polyanionic sugars, was able to block the paracrine mitogenic effects of growth factors released from tumor cells. Subsequently, a series of polyanionic naphthalene sulfonate derivatives of distamycin A were synthesized (Biasoli et al., 1993). These compounds are characterized by a common skeleton of four methylic-pyrrolic rings on a naphthalene ring, but vary in the position of the $-SO_3$ groups. Among these, PNU145156E (Fig. 4) was shown to be active in inhibiting the binding of bFGF to its cell surface receptor in vitro (Ciomei et al., 1994). These findings were confirmed by in vivo experiments showing a drug-induced inhibition of neovascularization and proliferation of solid tumors (Sola et al., 1995). In addition, using an in vitro model of two human N-SCLC cell lines, we demonstrated that this compound is able to counteract IGF-I binding to its transmembrane type-I receptor (de Cupis et al., 1997). Binding studies revealed an absence of variation in K_d values and in growth factor binding by drug pretreatment, as well as a decrease in ligand availability following coincubation of radiolabeled IGF-I and PNU145156E. These findings led us to suggest that the compound does not compete directly at the receptor level, but interferes with the growth factor/receptor interaction by capturing the mitogenic peptide itself and preventing its binding. Furthermore, the observation that the polyanionic naphthalene sulfonate distamycin A derivative was unable to dissociate a preformed growth factor/receptor complex upholds the hypothesis that polyanionic drugs bind preferentially to the mitogenic peptides, rather than to their related receptors (de Cupis et al., 1997).

B. Growth Factor Receptor Neutralization

Several strategies seeking to block growth factor/receptor binding are currently under investigation. One approach entails the use of antibodies able to bind to the receptor and thereby prevent growth factor interaction with binding sites. The up-regulation of growth factor receptors in many malignant tumors, which could also be associated with poor prognosis, represents a valid reason for considering growth factor receptors as therapeutic targets. In particular, because many tumors express high levels of EGF-R, a number of antibodies that block this receptor have been developed. These include the monoclonal antibody (MoAb) 225, which is believed to interact with sites near enough to the ligand binding site to counteract the binding process. The earliest studies showed that MoAb 225 treatment induced growth inhibition of human cancer cell lines, as well as of xenograft tumor models (Harris et al., 1992; MacDonald and Habib, 1992; Baselga et al., 1993; Scher et al., 1995). In addition, results showed that anti-EGF-R antibodies were able to enhance the cytotoxic effect of doxorubicin in A431 squamous cell carcinoma and breast cancer xenografts (Baselga et al., 1993). Moreover, other studies revealed that cisplatin and paclitaxel, in association with anti-EGF-R antibody, had at least additive effects on cell death (Hanauske et al., 1987; Aboud-Pirak et al., 1988). Slovin et al. (1996), on the basis of in vitro, in vivo, and immunohistochemical data, proposed the development of an MoAb 225-doxorubicin combination in patients with progressive androgen-independent prostate cancer.

For more than a decade reports have shown that c-erbB-2/Her-2 extracellular ligand-binding domain is elevated above control levels in the serum of patients with a variety of cancers, including prostate, colon, pancreatic, bladder, lung, ovarian, and gastric. Elevated serum c-erbB-2/Her-2 levels in these cancers are associated with tumor burden and metastatic disease. The quantitation of Her-2/*neu* extracellular ligand-binding domain levels will become important in these epithelial cancers especially as novel anti-c-erbB-2/Her-2 therapies are developed. Patients with c-erbB-2/Her-2 overexpression exhibit a reduced response to conventional treatments. Patients with estrogen receptor-positive/c-erbB-2/Her-2-positive metastatic breast cancer are less likely to respond to hormone treatment, and the survival duration is shorter than estrogen receptor-positive/c-erbB-2/Her-2-negative patients (Leitzel et al., 1995). Circulating levels of extracellular domain of the Her-2/c-neu-related protein could predict the response to antiestrogen therapy in advanced breast cancer patients (Yamauchi et al., 1997). Therefore, new therapeutic approaches targeting the cells overexpressing this protein and based on monoclonal antibodies have been developed. Binding of specific MoAbs to the extracellular domain of Her-2/*neu* inhibit tumor proliferation both in vitro and in vivo (Kita et al., 1996; Kopreski et al., 1996; Wright et al., 1997).

One of these MoAb, targeted to the c-erB-2/Her-2 receptor, is Herceptin (Transtuzumab), which is now approved for breast cancer treatment. A phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185$^{c\text{-}erbB\text{-}2/Her\text{-}2}$ monoclonal antibody with CDDP in patients with c-erbB-2/Her-2-overexpressing metastatic breast cancer refractory to CDDP-based chemotherapy treatment has been carried out. It

has been shown that the use of the MoAb in combination with the antineoplastic drug in that kind of patient results in objective clinical response rates higher than those previously reported for CDDP or the antibody alone and without increases of toxicity (Pegram et al., 1998). Several other studies, not yet published, regarding applications of Herceptin in the major human solid tumors, mainly breast cancer, are ongoing: 1) analysis of response of Herceptin plus taxol in c-erbB-2/Her-2-overexpressing and non-overexpressing metastatic breast cancer; 2) a phase II study with the combination Herceptin-vinorelbine as second line therapy for c-erbB-2/Her-2-positive metastatic breast cancer; 3) biochemical properties of Herceptin as inhibitor of c-erbB-2/Her-2 cleavage in breast cancer cells; 4) comparison response to Herceptin with that to other antibodies in detecting low levels of c-erbB-2/Her-2 overexpression; 5) a phase II study of Herceptin plus low-dose taxol in heavily antracyclines/taxanes/navelbine-pretreated breast cancer patients with Her-2/*neu* overexpression; 6) Herceptin administered as a single agent after chemoendocrinotherapy. Furthermore, in a case report, a complete response using Herceptin in a premenopausal woman with Her-2/*neu*-positive metastatic, heavily pretreated and chemoresistant breast cancer, has been described. Preliminary experiences of incorporating anti-c-erbB-2/Her-2 MoAb (Herceptin) in the chemotherapeutic armamentarium for breast, lung, and other solid tumors have been described as well. Finally, founded on the rationale that overexpression of c-erbB-2/Her-2 signaling may be among the causes of antiestrogen resistance in human breast cancer, a combination of anti-c-erbB-2/Her-2 antibody with pure antiestrogen ICI 182,780 has been successfully tested. In particular, the growth inhibitory effect of the antihormonal agent in cancer cells expressing both high levels of estrogen and c-erbB-2/Her-2 receptors has been enhanced. The hypothesis that patients with malignant solid tumors having high overexpression of c-erbB-2/Her-2 might benefit from adding Herceptin in the course of anticancer treatment has been successfully verified several times recently. The rationale is that because the efficacy of adding the antibody has already been verified in patients with metastatic breast cancer, it is likely that Herceptin may also work on other types of solid tumors. However, laboratory studies report that native anti-c-erbB-2/Her-2 MoAb causes only weak growth inhibition of human breast and ovarian cancer cell lines (Kita et al., 1996). Conversely, a greater reduction of cellular proliferation was achieved when antibodies were conjugated with toxins or radionuclides (Crews et al., 1992; De Santes et al., 1992; Tecce et al., 1993). Dean et al. (1998) recently suggested that several features of immunotoxin-mediated cell kill properties observed in vitro could prove relevant to the design of clinical studies for serotherapy in cancers.

C. Protein Tyrosine Kinase Inhibitors

Because protein tyrosine kinases (PTKs) catalyze the phosphorylation of tyrosine residues on target proteins, inhibitors of this process (which is absolutely necessary for signal transduction) would be expected to be effective in inhibiting receptor tyrosine kinase activity. In addition, nonreceptor PTKs, such as *src* family members, may be critically involved in cellular proliferation. In the past 15 years, many kinase inhibitors have been discovered and investigated (Levitski and Gazit, 1995; Klohs et al., 1997; Traxler, 1997; Boschelli et al., 1998; Lawrence and Diu, 1998). These compounds are small molecules (MW $<$ 1,000) of both synthetic and natural origin. Inhibitors of PTKs can be classified into those that compete for the ATP binding site (Langdon and Smyth, 1995; McMahon et al., 1998) and those that compete for the substrate binding site (Langdon and Smyth, 1995; Levitski and Gazit, 1995).

Several bioflavonoids, such as quercetin and genistein, are known to be competitive inhibitors of ATP binding to PTKs, resulting in growth inhibition. In particular, genistein, an isoflavone abundant in soy products, has been shown to counteract proliferation of breast and prostate cancer cells (Peterson and Barnes, 1993, 1996; Clark et al., 1996; Wang et al., 1996). Knowing that *ras* function represents a convergence point for signaling through both receptor-tyrosine kinase and *src* family kinases, Clark et al. (1996) investigated the effects of genistein on proteins that regulate this process. In particular, these authors evaluated the activity of the bioflavonoid on Shc and Grb-2, two proteins involved in controlling *ras* function. Shc is tyrosine-phosphorylated by *src* family kinases and by activated growth factor receptors, whereas Grb-2 mediates signal transduction from activated growth factor-receptors through *ras*. Clark and colleagues showed that genistein-induced inhibition of breast cancer cellular proliferation is accompanied by decreased Shc tyrosine phosphorylation and decreased association between Shc and Grb-2 SH2 domain, together with an inhibition of MAP kinase activity (Clark et al., 1996). Furthermore, the effects of genistein are somewhat more complicated, in that in addition to being a tyrosine kinase inhibitor, this compound counteracts the activity of other enzymes, such as protein kinase C and topoisomerase II (Clark et al., 1996). Wang et al. (1996) demonstrated that long-term exposure of the estrogen-receptor-positive MCF-7 breast cancer cell line to genistein resulted in an antiestrogenic response, as indicated by a down-regulation of estrogen receptor mRNA levels and by an attenuated cellular response to estradiol treatment. In light of findings that antiestrogens are able to increase TGF-β1 expression (Perry et al., 1995), Sathyamoorthy et al. (1998) investigated whether genistein had any effect on TGF-β1 mRNA levels in both normal and malignant breast cancer cells. The compound was shown to exert a differential effect, causing a dose-dependent increase in TGF-β1 mRNA levels only in normal epithelial mammary cells. This stimulation of growth factor expression could be responsible, at least in part, for the observed cell growth inhibition and induction of apoptosis. One possible explanation for the resistance of MCF-7 breast cancer

cells to the growth inhibitory effects of genistein is that these cells metabolize the compound differently from normal epithelial cells. It is well known that TGF-β functions as a growth inhibitor in mammary epithelial cells; this understanding underlies the recent interest in the use of agents able to modulate production of this growth factor in chemoprevention strategies for breast cancer (Koli and Keski-Oja, 1995). In keeping with these findings, a number of reports hold that the increased consumption of soy-based foods, which are rich in genistein, is associated with lowered incidence of hormone-dependent cancers (Adlercreutz et al., 1991; Lee et al., 1991; Murrill et al., 1996).

Erbstatin, a natural product isolated from Streptomyces cultures, is a PTK inhibitor that competes at the substrate site (Umezawa et al., 1986; Bishop et al., 1990; Takeura et al., 1991). In addition, a demonstrated activity of erbstatin against protein kinase-C suggests its limited selectivity for PTKs (Bishop et al., 1990). Nevertheless, erbstatin provided the rationale for the development of a series of synthetic PTK inhibitors, known as tyrphostins (Yaish et al., 1988; Levitski and Gilon, 1991), which are chemically characterized by the benzylidene-malononitrile structure. The tyrphostins thus far developed have relatively high specificity for the PDGF-R and the EGF-R (Yaish et al., 1988; Bilder et al., 1991). Karnes et al. (1998) recently investigated the effects of a selective EGF-R tyrosine kinase inhibitor, PD 153035, on colon cancer cell lines to examine the potential application of EGF-*R*-targeted treatment. These authors observed a drug-induced cytostatic effect on cellular proliferation at concentrations able to block EGF-R autophosphorylation, but observed classical features of apoptosis when higher concentrations were used. Although the programmed cell death appeared to be independent of p53 induction and to be associated with activation of caspase 3-like proteases, the mechanism whereby the tyrosine kinase inhibitor induces apoptosis is still unclear.

D. Phosphotyrosine Phosphatase Activators

Although phosphorylation of tyrosine residues by tyrosine kinases is a crucial event in triggering growth factor signal transduction pathways, the dephosphorylation step, catalyzed by phosphotyrosine phosphatases (PTPases), switches off this biochemical process. Compounds that are able to activate PTPases and thereby inhibit cellular proliferation include antiestrogens and somatostatin.

Nonsteroidal and steroidal antiestrogens (Fig. 5) modulate breast cancer cellular proliferation not only by acting as estrogen antagonists, but also by interfering with growth factor signaling pathways (see also following paragraph and de Cupis and Favoni, 1997). Antigrowth factor activity occurs in the absence of active estrogen and is accompanied by a drastic reduction in the expression of several growth factor-mediated responses (Katzenellenbogen and Norman, 1990; Philips et al., 1993; Freiss and Vignon, 1994). Published findings show that the inhibition of breast cancer proliferation stimulated by growth factor is associated by a concomitant increase in membrane PTPase (Freiss and Vignon, 1994). The increase in enzyme activity was selectively achieved by nuclear estrogen antagonists, the nonsteroidal 4-hydroxy-tamoxifen, and the "pure" antiestrogens ICI 164,384 and ICI 182,780 (Fig. 5), which are able to counteract growth factor-induced cellular proliferation (Freiss and Vignon, 1994; Freiss et al., 1998). Conversely, progesterone receptor ligands (progestins and antiprogestins) that antagonize estrogenic activity (Vignon et al., 1987) did not show any antigrowth factor activity (Freiss et al., 1990) and failed to stimulate PTPase activity (Freiss and Vignon, 1994).

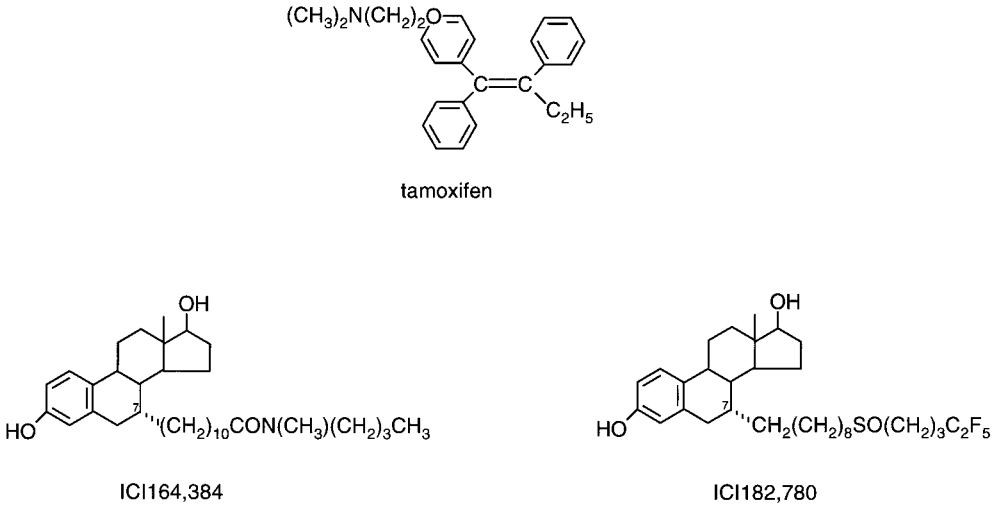


FIG. 5. Nonsteroidal (tamoxifen) and steroidal (ICI 164,384 and ICI 182,780) antiestrogens inhibit cell signaling by turning off the receptor phosphorylation process and/or by counteracting the estrogen-mediated growth factor and receptor synthesis. ICI 182,780 differs from ICI 164,384 in two key features of the 7α side chain: the amide moiety of ICI 164,384 was replaced by a sulfinyl group, and the terminal alkyl function was fluorinated to reduce the potential metabolic attack.

Finally, the addition of a specific PTPase inhibitor (sodium orthovanadate) was shown to prevent antiestrogen-induced inhibition of cell growth, thus indicating that PTPases are crucial for the antiproliferative effects of these compounds.

Somatostatin is a recognized inhibitor of a wide range of biological activities, including cell proliferation (Schally, 1988). In mammals, somatostatin binds to at least five different subtype receptors (Weckbecker et al., 1993). Although natural somatostatins have short half-lives (≈3 min) and are rapidly degraded, somatostatin analogues have been used in the therapy of various human tumors (Schally, 1988; Bogden et al., 1990; Weckbecker et al., 1992, 1993; Radulovic et al., 1993; Anthony et al., 1994). As far as the intracellular mechanisms of somatostatin and its analogues are concerned, the modulation of PTPase activity is held to be one of the main pathways responsible for the drug-induced inhibition of cell growth. In particular, the somatostatin-dependent increase in PTPase activity was shown to lead to the dephosphorylation of EGF-R, resulting in the inhibition of the growth factor-induced proliferative activity (Pan et al., 1992).

E. Inhibition of Growth Factor and Growth Factor Receptor Synthesis

The fact that estrogens can influence the synthesis and secretion of growth factors and their receptors implies an indirect mechanism of action of steroids in stimulating cell growth. The blocking of and/or interference with these biochemical pathways could represent a new means for a pharmacological approach, especially to breast cancer therapy. Antiestrogens achieve this goal by competing with estradiol for binding to the estrogen receptors through which intracellular hormone effects are mediated.

Tamoxifen (Fig. 5), a triphenylethylene derivative, is the molecule that has undergone the most extensive clinical evaluation and that represents the treatment of choice for the endocrine management of breast cancer. Several experimental studies were performed to evaluate the effect of tamoxifen and its active metabolite, 4-hydroxy-tamoxifen, on growth factor-mediated cell growth. Berthois et al. (1989) published data from studies carried out to establish the regulation of EGF-receptor by estrogen and 4-hydroxy-tamoxifen in the MCF-7 breast cancer cell line. They demonstrated that the hormone and the drug modulate the receptor level through opposite mechanisms. In addition, 4-hydroxy-tamoxifen was shown to induce a decrease in IGF-I binding sites, thus providing a possible explanation for the reduced IGF-I mitogenic effect observed after the drug treatment (Freiss et al., 1990). Moreover, clinical research shows that tamoxifen administration is associated with a reduction of serum IGF-I concentration (Pollak et al., 1992). Huynh et al. (1993), using an in vivo experimental system, reported that tamoxifen inhibits the expression of the IGF-I gene in common target organs for breast cancer metastasis. Moreover, down-regulation of IGF-I receptor autophosphorylation is documented (see previous section). Nevertheless, it is well known that although tamoxifen and 4-hydroxy-tamoxifen compete efficiently for the estrogen receptor, they retain agonist activity both in vitro and in vivo. In addition, the use of these compounds is limited by the possible development of drug resistance.

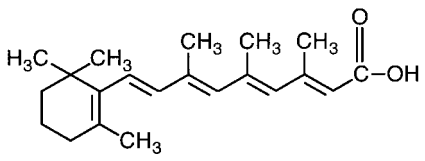
To overcome these drawbacks, molecules with steroid-like structure, conceptually devoid of antiestrogenic activity, have been synthesized. Representative of these drugs, commonly identified as "pure" steroidal antiestrogens, are ICI 164,384 and ICI 182,780 which are characterized by an alkylamine side chain at the 7α-position of the B ring in the steroid (Fig. 5). In particular, the presence of an alkyl side chain at the C7 position of the steroid nucleus makes these compounds able to interfere with the estrogen receptor dimerization function, possibly by steric hindrance. This would be consistent with the finding that the length of the side chain at position 7 is critical in the activity of this family of steroids (reviewed in de Cupis and Favoni, 1997). With respect to interaction with estrogen receptors, steroidal antiestrogens are thought to bind to form an antiestrogen-estrogen-receptor complex that either does not bind to the estrogen responsive elements or, if DNA binding does occur, is unable to promote gene transcription and consequently any manifestation of estrogen action. Moreover, Parker (1993) showed that the novel antiestrogens, in contrast to 4-hydroxy-tamoxifen, increase estrogen receptor turnover and suggested that this phenomenon is a consequence of the impaired dimerization described above.

With respect to the ability of these steroidal antiestrogens to modulate growth factor activity, we reported data concerning the action of ICI 182,780 in controlling IGF-I-controlled breast cancer cell growth (de Cupis et al., 1995). As a model system, we used several breast cancer cell lines whose features represent typical characteristics of breast cancer in patients. The steroidal antiestrogen was much more potent than 4-hydroxy-tamoxifen in inhibiting the IGF-I-stimulated cellular proliferation. These data agree with those obtained by Wakeling (1990) showing that ICI 164,384 is able to down-regulate the growth factor-controlled MCF-7 cell growth. In our hands, the observed growth inhibition could also be partially related to the ability of ICI 182,780 to reduce the number of binding sites for the mitogenic peptide. Huynh et al. (1996) recently reported that the antiproliferative effect of ICI 182,780 is related, at least in part, to the up-regulation of IGF-BP3 gene expression induced by the steroidal antiestrogen.

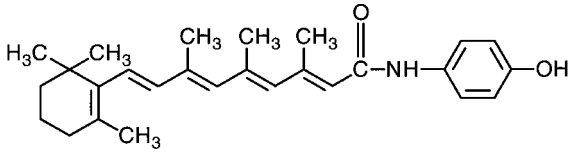
Regarding clinical applications, DeFriend et al. (1994) reported the results of a trial conducted on 56 women with primary breast carcinoma to verify ICI 182,780 tolerance, pharmacokinetics, and short-term biological

effects. They found that this novel compound was well tolerated after short-term administration and produced evident antiestrogenic effects in human breast tumors without showing evidence of agonistic activity. Finally, treatment with ICI 182,780 in 19 patients with advanced tamoxifen-resistant breast cancer was shown to yield a 69% response rate (Howell et al., 1995).

Retinoids are a pharmacological class consisting of vitamin A (retinol) and related derivatives that normally play a critical role in growth, reproduction, epithelial cell differentiation, and immune function. These compounds, such as retinoic acid (Gottardis et al., 1996) and *N*-(4-hydroxyphenyl)retinamide (4-HPR) (Favoni et al., 1998) (Fig. 6), also have efficacy as antineoplastic agents through interference with growth factors and/or their receptors synthesis at the cellular level. These agents act by regulating gene expression through receptors belonging to the steroid/thyroid superfamily (Giguere, 1994). Reports show that retinoid-induced inhibition of breast cancer cellular proliferation is associated with a reduction in expression of estrogen-responsive genes, such as TGF-α (Fontana et al., 1992). An experimental study carried out in our laboratory (Favoni et al., 1998) indicated that 4-HPR was able to abolish the mitogenic effect of exogenous IGF-I on MCF-7 breast cancer cell line. In addition, we found that the synthetic retinoid is able to down-regulate the binding of IGF-I to its cell surface receptor. This interference was due to the 4-HPR-mediated inhibition of type-I IGF receptor gene expression, as confirmed by the observed decrease of IGF-I receptor mRNA. In keeping with the demonstration that IGF-I receptor protects tumor cells from apoptosis (Resnicoff et al., 1995), the significant induction of programmed cell death that we observed after 4-HPR treatment in our in vitro model, could be related, at least in part, to the loss of type-I IGF cell surface receptor.



retinoic acid



N-(4-hydroxyphenylretinamide)

FIG. 6. Retinoids, a pharmacological class consisting of vitamin A and related derivatives such as retinoic acid and 4-HPR, have been recognized as inhibitors of cellular proliferation. Among the proposed mechanisms of their action is the ability to interfere with growth factor and receptor synthesis.

F. Farnesyltransferase Inhibitors

Farnesyltransferase is the enzyme that transfers farnesyl isoprenoid to certain cell membrane associated proteins. Pharmacological inhibitors of this enzyme were designed as a strategy to counteract *Ras*-dependent tumors, which require *Ras* farnesylation to function. Different structural classes of farnesyltransferase inhibitors (FTIs) have shown specificity, potency, cell penetration, and lack of toxicity (Gibbs and Oliff, 1997; Leonard, 1997; Sebti and Hamilton, 1997). In in vitro models, FTIs are able to inhibit the anchorage-independent proliferation of *Ras*-transformed cells (Garcia et al., 1993; James et al., 1993; Kohl et al., 1993). In addition, and in association with the loss of anchorage-independent potential, FTI-treated cells flatten, enlarge, and acquire the morphologic and growth regulatory characteristics of nontransformed parental cells (James et al., 1994; Prendergast et al., 1994). FTIs were shown to inhibit prostate cancer cellular proliferation in vitro and clonogenicity in soft agar (Slovin et al., 1996). Consistent with their in vitro effects, FTIs block tumor formation in mouse xenograft models (Sun et al., 1998). Furthermore, in all in vivo studies described to date, these compounds have proven to be basically nontoxic.

Unexpectedly, recent investigations suggest that inhibition of *Ras* farnesylation is neither necessary nor sufficient for the desired antineoplastic activity of FTIs, thus indicating that *Ras* may be an irrelevant target. In particular, FTIs were observed to inhibit proliferation of cells transformed with oncogenic Ras proteins engineered to function independently of farnesylation (Lebowitz et al., 1995). Moreover, the susceptibility of human tumor cell lines to FTIs is not correlated with *Ras* mutation status (Sepp-Lorenzino et al., 1995). Thus, these observations imply that FTIs target farnesylated proteins other than Ras. Rho proteins are small GTPases that, like Ras, have to be isoprenylated to function. These proteins regulate cytoskeletal actin organization and adhesion, as well as proliferation (Symons, 1996; Tapon and Hall, 1997; Van Aelst and D'Souza-Schorey, 1997). Lebowitz and Prendergast (1995) reported that RhoB, the only Rho protein regulated by growth and stress stimuli (Jahner and Hunter, 1991; Fritz et al., 1995; Zalcman et al., 1995), is a target of FTIs. RhoB protein is either farnesylated or geranylgeranylated in cells. Although FTI treatment causes a rapid depletion of the farnesylated form of RhoB (RhoB-F), geranylgeranylated forms of RhoB (RhoB-GG) accumulate because the drugs do not counteract geranyltransferase-I activity. Elevation of RhoB-GG level is sufficient alone to mediate many FTI-induced effects, including inhibition

of cellular proliferation (Lebowitz et al., 1995, 1997a; Du et al., 1999). Moreover, recent findings reveal that FTI-induced depletion of RhoB-F is important for apoptosis that occurs when cellular substratum attachment is denied (Lebowitz et al., 1997b). Importantly, even though FTIs seemed to cause complete tumor regression in mice, cessation of FTIs treatment led to a rapid recovery of tumor growth (Kohl et al., 1995). Thus, tumor persistence, if also observed in patients, would require long-term FTIs treatment that could increase the likelihood of side effects and drug resistance. In addition, in light of findings on the incomplete elimination of the tumor, a key future line of research might focus on the involvement of FTIs in suppressing the expression of angiogenic growth factors. Although further validation is necessary, FTIs might also act by blocking Rho-dependent overexpression of VEGF and, therefore, tumor vascularization (Prendergast, 1999). A recent study (Prendergast, 1999) reports that activation of the PI3K pathway by IGF-I is able to counteract FTIs cytotoxicity. Preclinical data suggest that drug activity may be enhanced by combining FTIs with signal transduction inhibitors that affect growth factor/PI3K signaling (Bernhard et al., 1996, 1998).

G. Antisense Oligonucleotide Strategies

As extensively described, overexpression of EGF-R is observed in many human tumors, including most glioblastomas, breast, lung, ovarian, colorectal, and renal carcinomas (Salomon et al., 1995; Kunkel et al., 1996). Moreover, elevated EGF-R levels are associated with poor prognosis. These findings provide the grounds for cancer therapy approaches based on blocking the activity of this receptor. In addition to anti-EGF-R antibodies and drugs able to inhibit the receptor tyrosine kinase activity (see previous sections), antisense oligonucleotides appear to be promising tools for tumor treatment. Antisense oligodeoxynucleotides inhibit gene expression in a selective and targeted sequence-specific manner (Agrawal, 1992; Stein and Cheng, 1993; Wagner, 1994; Robinson et al., 1996) and are known to counteract growth in several human carcinoma cell lines (Neckers et al., 1992; Normanno et al., 1996). Furthermore, the efficacy of an antisense compound in inhibiting the in vivo growth and metastases in malignant pituitary tumors is documented. Witters et al. (1999) recently identified several oligonucleotides that inhibit the expression of EGF-R mRNA in human lung and ovarian carcinoma cell lines. The antisense compounds used by these authors also significantly down-regulate EGF-R production and in vitro cell growth.

IV. Concluding Remarks

Antineoplastic pharmacology research is currently enjoying a propitious period in the wake of advances allowing the set up of selective therapies based on the biological and biochemical differences between normal and malignant cells. Furthermore, the exponentially increasing knowledge of the pathogenetic mechanisms involved in tumor cell growth has led to the introduction of a more mechanism-based compound screening; this can be achieved either by evaluating new targets as preferential sites for anticancer drug treatment or by using previously known targets, such as growth factors, in a more sophisticated way.

Growth factors are polypeptide molecules that regulate cell proliferation by binding to their specific high-affinity receptors in the plasma membrane, thus stimulating receptor-mediated activation of intracellular signal transduction pathways. An impressive body of evidence accumulated over the past decade, derived not only from empirical observations but also from experimental studies, clearly indicates that such biochemical regulatory pathways of several classes of growth factors are active and play a critical role even in the progression of human malignancy.

New anticancer approaches have been designed to translate these findings into practical applications. Many therapies against growth factors, their receptors, and carrier proteins have been explored, with promising success, in a variety of experimental in vitro systems. Agents can interact with polypeptides contributing to malignant behavior and either inactivate them or block the binding to their receptors. Antibodies directed against specific growth factors can inhibit growth factor-dependent tumor growth. Growth factor receptor antibodies, alone or toxin-conjugated, can inhibit or destroy tumor cells. Growth factor fragments able to block their cognate receptor binding site may have growth inhibitory activity. Interference with a signal transduction cascade triggered by growth factor-receptor binding could deactivate the effect of the ligand itself (e.g., tyrosine kinase inhibitors or tyrosine phosphatase activators). Novel "pure" steroidal antiestrogens can inhibit the synthesis of such growth factors and receptors. The antisense oligonucleotide strategy may be used to down-regulate the production of translated products such as mitogenic factors and receptors. The need for *ras* and/or *Rho-B* to be farnesylated in order to function suggests the design of pharmacological inhibitors of farnesyltransferase to counteract *ras* (*Rho-B*)-dependent tumors.

Most of the described approaches are not intended to be mutually exclusive; rather, their articulate biopharmacological combination could allow successful therapeutic options to kill different tumoral cell populations in different cancer patients, or in the same kind of tumor of any single patient. However, it must borne in mind that even this new generation of anticancer therapies must overcame several hurdles. Drug resistance will likely be faced and, above all, promising findings obtained in vitro or in experimental animal systems will

have to be carefully assessed because they often yield disappointing clinical results.

In conclusion, the noteworthy advances already made by translating concepts of modern molecular oncology into therapeutic applications provide stimulating alternatives to conventional therapies for some cancers.

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