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Review Article

Protein Kinases as Therapeutic Targets

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Protein kinases and phosphatases are likely targets for the development of therapeutic drugs since they are involved in specific signaling pathways which regulate cell functions such as metabolism, cell cycle progression, cell adhesion, vascular function and angiogenesis. Protein phosphorylation and dephosphorylation serve as molecular switches for modulating these processes and the level and duration of each is a highly regulated process in normal cells. Several compounds that inhibit the activity of tyrosine kinases are being evaluated as cancer therapeutic agents in clinical trials. Diabetes and complications of diabetes also involve deregulated levels of protein kinases. New approaches for regulating kinase gene expression include specific antisense oligonucleotides for inhibiting post-transcriptional processing of the messenger RNA, naturally occurring products and their chemical derivatives to inhibit kinase activity, and monoclonal antibodies to inhibit receptor linked kinases. Inhibition of phosphatases also serves to alter the duration of phosphorylation by kinases. Considerations for development of effective inhibitors include non-specific actions of compounds, cellular uptake, multiple intracellular targets that can dilute the effective cellular concentration of an agent, and tissue specificity. Kinase inhibitors may allow other therapeutic agents additional time to become effective and they may act synergistically with current treatments.

KEY WORDS: protein kinase; signal transduction; drug design; chemotherapeutic agents.

Eukaryotic protein kinases constitute a large family of homologous proteins that catalyze the transfer of the gamma phosphate group of ATP or GTP to the hydroxyl group of serine, threonine or tyrosine in a substrate protein. Protein kinases differ in structure, subcellular location, substrate specificity, and function. Cellular signaling cascades rely on the phosphorylation status of pathway proteins to alter their function. Some substrates transmit the signal, while the final protein targets are altered in activity. Phosphorylated serine, threonine, or tyrosine residues are substrates for specific protein phosphatases so that phosphorylation and dephosphorylation serve as molecular switches and each is highly regulated as to level and duration (Figure 1) (1–4).

Cellular functions such as gene expression, cytoskeletal integrity, cell adhesion, cell cycle progression, and differentiation are controlled by the complex interplay of protein kinases and phosphatases in specific signaling pathways (5–12). Malfunctions of cellular signaling have been associated with many diseases including cancer and diabetes. Regulation of signal transduction by cytokines and the association of signal molecules with protooncogenes and tumor suppressor genes have been subjects of intense research in the industrial setting as well as in academics. Many therapeutic strategies can now be developed through the synthesis of compounds which activate or inactivate protein kinases. In a multicellular organism, intercellular communication plays a crucial role under normal as well as pathological conditions. Coexistence of abnormal cells with normal cells provide the stroma and blood supply essential for maintaining growth and progression of tumors. Such codependence relies on a wide array of receptors and signal transduction pathways to the nucleus of either the host or cancer cell. Since aberrant expression/activation of protein kinase C appears to be involved in the development of certain types of cancer, diabetes and complications of diabetes, the search for selective PKC inhibitors is a major goal of many researchers. Mutant tyrosine kinases are also often associated with carcinogenesis in certain organs, making tyrosine kinase signaling pathways attractive targets for oncology research.

Cytokines, hormones and growth factors bind and activate specific receptors. The molecular mechanisms of signal transduction pathways were elucidated by identifying the specific protein kinase cascades along with their downstream targets, which include some specific transcription factors. Protein kinases act in concert with cytokines, cell cycle regulatory molecules, proteins of apoptotic machinery and transcription factors via pathways that regulate cell metabolism, differentiation, proliferation and death. Many therapeutic strategies are aimed at critical components in signal transduction pathways (9–13).

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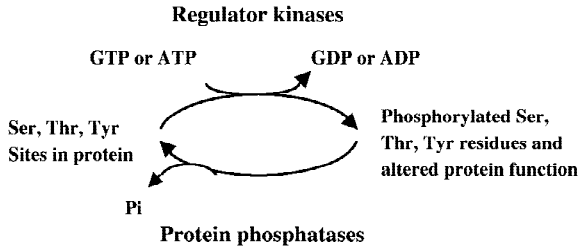

Fig. 1. Kinases and phosphatases provide molecular switches for altering protein function. Protein phosphorylation is a post-translational modification in cells that is reversible by the action of phosphatases. It is one of the most important mechanisms used by cells for signal transduction.

PROTEIN KINASES ARE GROUPED FUNCTIONALLY

Eukaryotic protein kinases typically encode a 250 amino acid catalytic domain that is commonly under the control of a separate regulatory domain or subunit. Hanks and Hunter have classified them on the basis of, their structural and functional properties (14). The protein kinase "phylogenetic tree" (Figure 2) was derived from aligning kinase domain amino acid sequences (14). There are five kinase categories: 1) the cyclic nucleotide-regulated and phospholipid-regulated kinases and ribosomal S6 kinases (AGC), 2) the Ca^{2+}/calmodulin kinases (CaMK), 3) the cyclin-dependent kinases (CMGC), 4) the protein tyrosine kinases (PTK), and 5) "other" kinases falling outside the four major groups. Members of groups share substrate preferences. For example, both the AGC and CaMK groups phosphorylate serine/threonine residues near arginine and/or lysine. Members of the serine/threonine kinase group, CMGC, phosphorylate serine/threonine in proline-rich domains. The CMGC kinases have larger catalytic domains than other kinases. The PTK group includes both receptor and non-receptor kinases that phosphorylate tyrosine residues. Other kinases can phosphorylate either serine/threonine and tyrosine residues and some are termed dual-specificity kinases. The cellular function of many kinases was elucidated initially by broad specificity inhibitors. With the rapid expansion of DNA sequence databases, it is likely that additional kinases will be discovered.

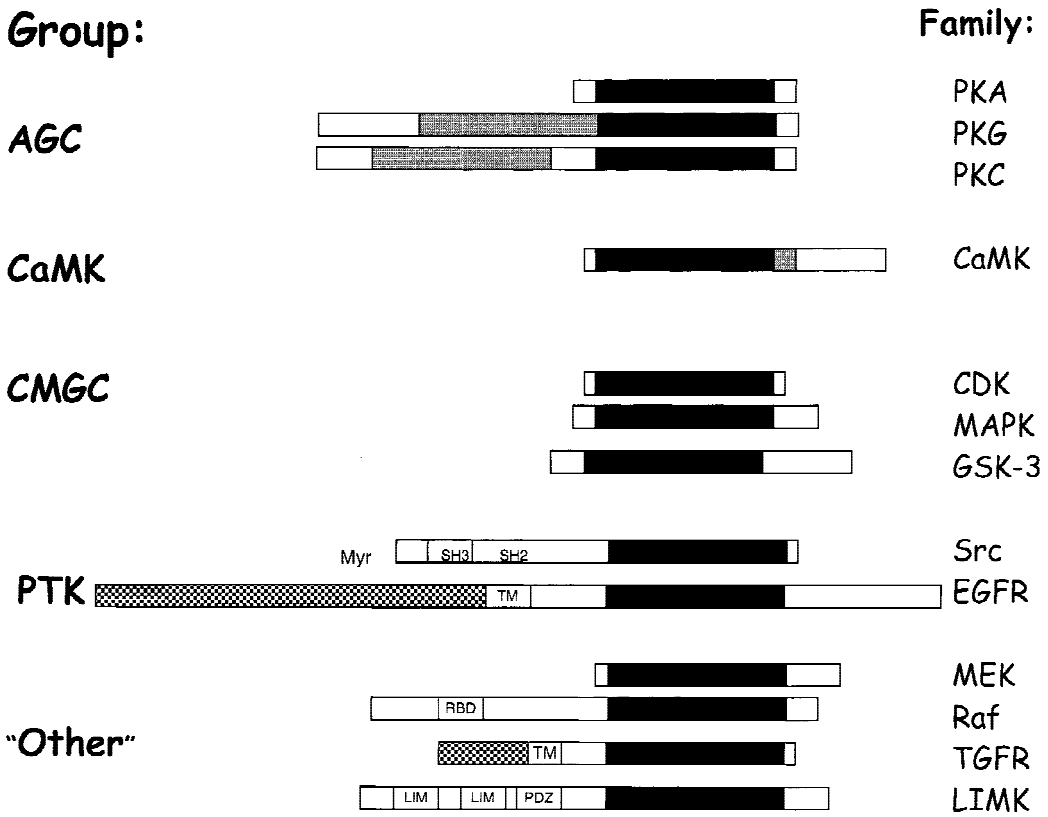

Fig. 2. Diagram of the major families of protein kinases. Kinase catalytic domains are shaded black. The AGC group includes cyclic nucleotide-dependent protein kinases (Protein kinase A (PKA) and Protein kinase G (PKG)) and lipid-dependent protein kinase C (PKC) families. The catalytic subunit of PKA is shown. PKG contains a cGMP binding domain which is shaded gray. The regulatory domain of PKC is also shaded gray. The CaMK group shows the shaded autoregulatory domain (site that binds Ca2+/calmodulin). The CMGC group includes the CDK, MAPK/ERK and GSK-3 families. The PTK group includes the Src family and the EGFR RTK family. The N-terminal myristoyl (Myr) modification allows for membrane attachment. The SH2 and SH3 domains are shown. RTKs contain transmembrane (TM) and extracellular binding domains for ligands (checked shading). The "other" group includes MEK, Raf, and the TGF-β receptor and LIM kinases. Raf kinases have a Ras binding domain (RBD). The TGF-β receptors have extracellular ligand binding domains (checked shading) and TM domains. The LIM and PDZ domains are noted in LIMK.

DEPHOSPHORYLATION BY PROTEIN PHOSPHATASES

Dephosphorylation by protein phosphatases plays an equally important role in regulating cellular processes. Protein phosphatases have specificities that are as distinct as those of the protein kinases, and a similar number of genes encode both family members (15,16). They are classified based on substrate specificity, dependence on metal ions, and sensitivity to inhibitory agents. Table 1 summarizes the distribution and known inhibitors of protein phosphatases which have been demonstrated to play a role in signal transduction. They possess a 230 amino acid catalytic domain and contain a number of regulatory subunits that govern subcellular localization and enzymatic activity (15,20). The activities of PP1 and PP2A are independent of metal ions (15,16). The catalytic subunit of PP1 binds to regulatory subunits that determine PP1 subcellular localization and activity (17) while PP2A is inactivated by transient phosphorylation of tyrosine residues on the molecule (18). PP2B, also known as calcineurin, consists of a catalytic subunit (A-subunit, 6kDa) and a regulatory subunit (B-subunit, 19kDa). It is dependent on the Ca^{2+}-calmodulin complex for complete activation (19). Over 40 protein tyrosine phosphatases (PTP) have been characterized. Specific activators of protein phosphatases are still being sought. The C2, C6 and C16 ceramides are reported activators of protein phosphatases (21–23).

THERAPEUTIC STRATEGIES FOR TREATING CANCER

Cancer treatment strategies include: (i) inhibiting tumor cell proliferation, (ii) inducing tumor cell death by necrosis or apoptosis, (iii) inhibiting tumor angiogenesis, (iv) facilitating host immune system, (v) inducing vegetative tumor cells to undergo terminal differentiation, (vi) inhibiting metastases by inhibiting tumor cell adhesion and invasiveness of normal tissues.

Table 1. Protein Phosphatases Involved in Signal Transduction

Protein phosphatase type	Subcellular distribution	Known inhibitors
PP1	Cytosol	Calyculin A
	Nucleus	Nodularin
	Myofibrils	Tautomycin
	Glycogen particles	
PP2A	Cytosol	Calyculins
	Nucleus	Microcystins
	Mitochondria	Nodularin
		Okadaic acid
PP2B (calcineurin)	Cytosol	Cyclosporin A
	Nucleus	FK506
	Plasma membrane	Immunophilin complexes
	Synaptosomes	Cypermethrin
		Deltamethrin
		Fenvalerate
PTP	Plasma membrane	bp V(phen)
	Nucleus	mpV(pic)
		Dephostatins
		Phenylarsine Oxide
		Sodium Orthovanadate

TYROSINE KINASES AS THERAPEUTIC TARGETS FOR CANCER CHEMOTHERAPY

Recent efforts in drug design have targeted specific kinases. Ras is one of the most frequently mutated oncogenes in human cancers (24,25), and Ras signaling is a downstream event of tyrosine kinase activation. Therefore modifiers of tyrosine kinases are actively being investigated as anti-cancer drugs. Cytoplasmic tyrosine kinases frequently contain SH2 and SH3 domains (*src* Homology 2 and 3 domains) which mediate intra- and interprotein interactions (Figure 2). SH2 domains bind to phosphotyrosine sites with flanking amino acids that are specific for the particular SH2 sequence, and SH3 domains latch on to proline-rich regions (10).

Receptor tyrosine kinases (RTKs), many of which are growth factor receptors, are transmembrane glycoproteins with a membrane spanning domain and a conserved cytoplasmic tyrosine kinase domain. The RTK superfamily consists of 18 families in vertebrates and includes 56 different receptors including insulin, fibroblast growth factor (FGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), epidermal growth factor (EGF), platelet derived growth factor (PDGF), and vascular endothelial growth factor (VEGF), and many other receptors.

There is considerable structural similarity among each RTK subfamily. Related receptors usually bind related ligands such as the HER family of receptors (EGF receptor, HER2, HER3 and HER 4) and their ligands (TFG-α), amphiregulin, heparin binding EGF (HB-EGF), betacellulin, and heregulin. Each of these receptors contains two domains that share the conserved sequence pattern of nearly 50 cysteine residues; additionally, each of the ligands contains a conserved motif of cysteine sextet present in the prototypical EGF (10).

Binding of a ligand to an RTK leads to receptor dimerization and activation of the intracellular catalytic (kinase) domain. In the dimer, the catalytic unit of one receptor subunit phosphorylates specific tyrosines in the other subunit. The phosphorylated receptors then phosphorylate or interact with other adapter and signaling molecules through phosphotyrosines, triggering a cascade of further phosphorylations and/or dephosphorylations. After a series of downstream events involving several proteins, the signal reaches the nucleus in the form of a molecule which can alter the activity of the genetic machinery to control cell proliferation, differentiation, cell metabolism, and even programmed cell death (apoptosis) (10,26,27).

Insulin-like growth factor 1 (IGF-1) and related receptors exist as preformed dimers of α and β chains. Following activation, the ligand-induced phosphorylation is similar to the RTK pathway. The phosphotyrosines of the receptor can bind to an adapter molecule or substrate such as phosphatidyl inositol (PI) 3-kinase. The association of PI-3-kinase with the intracellular domain of phosphorylated RTK enhances PI-3 kinase activity via allosteric activation of the catalytic subunit (10,28). In contrast to substrates of RTKs such as PI-3-kinase, the adapter molecules contain no intrinsic catalytic activity. An example of an adapter molecule in this signaling is Grb2 in the MAP kinase pathway (29).

There is strong evidence for the involvement of RTKs in human cancer making these a target for inhibition (10,30). Examples of these are *erbB (EGF receptor), neu* (HER2), *kit*

(stem-cell factor receptor), *fms* (CSF1 receptor), *met* (HGF receptor), *trk* (neurotrophin receptor), *sea, ros, ret, eyk,* and *axl* (10). A number of cytoplasmic tyrosine kinases including *src* and *abl* behave as oncogenes when mutated or inappropriately expressed (10). Nearly 30% of human breast and ovarian cancers show amplified expression of the receptor tyrosine kinase HER2 (31). Amplification of HER2 gene also correlates with decreased patient survival and a shorter time for recurrence of disease (32).

Blocking of the receptor/ligand interaction is also an effective therapeutic target. Herceptin (Genentech, San Francisco, CA) is a humanized monoclonal antibody against HER2. The success of Herceptin in cancer treatment supports the hypothesis that blocking certain RTKs can curtail cancer progression. Alteration or overexpression of RTKs such as PDGF and EGF receptors has also been associated with certain cancers. Inhibitors of RTKs may inhibit the growth and proliferation of such cancers, since RTKs stimulate tumor cell proliferation.

Inhibitors of RTKs are useful in preventing tumor angiogenesis and can eliminate support from the host tissue by targeting RTKs located on vascular cells (e.g., blood vessel endothelial cells and stromal fibroblasts (FGF receptor)). Another example of restricting blood supply to a tumor could be through vascular endothelial growth factor (VEGF) and its receptor. Several splice variants of VEGF are known (e.g., $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, $VEGF_{206}$) which vary in the number of amino acids in the peptide (33). VEGF stimulates endothelial cell growth during angiogenesis, and increases the permeability of tumor vasculature so that proteins and other growth factors become accessible to the tumor (10). Broad-spectrum antitumor efficacy of an oral dosage form of an inhibitor of VEGF signaling has been reported (33). Thus, inhibition of VEGF receptor signaling presents an important therapeutic target. An extracellular receptor can also be a target for inhibition. For example, the EGF receptor family and its ligands are overexpressed and exist as an autocrine loop in many tumor types. One EGF-related peptide, amphiregulin, is coexpressed in pancreatic and ovarian cancer (10). HB-EGF is expressed as an autocrine loop in gastric cancer. EGF receptor is found in over half of breast tumors unresponsive to hormone (10). EGF is found in many tumors, and EGF may be required for tumor cell growth. Antibody to EGF blocked the growth of tumor xenografts in mice (35). An antisense oligonucleotide for amphiregulin inhibited growth of a pancreatic cancer cell line (36). A variety of inhibitors of RTKs are listed in Table 2, some of which are already in clinical trials.

OTHER TARGET POSSIBILITIES

Many tyrosine kinase inhibitors are derived from natural products including flavopiridol, genistein, erbstatin, lavendustin A, staurosporine, and UCN-01. Inhibitors directed at the ATP binding site are also available (11,37). Signals from RTKs can also be inhibited at other target sites such as: nuclear tyrosine kinases, membrane anchors (inhibition of farnesylation) and transcription factors.

DEFINING THE TARGET

Targeting the signaling potential of growth promoting tyrosine kinases such as EGFR, HER2, PDGFR, *src,* and *abl,* will block tumor growth while blocking IGF-1 and TRK will interfere with tumor cell survival. Inhibiting these kinases will lead to tumor shrinkage and apoptosis. Flk-1/KDR and *src* are kinases necessary for neovascularization (angiogenesis) of tumors and inhibition of these will slow tumor growth thereby decreasing metastases (38). *Met* promotes cell migration, and inhibiting this kinase should also decrease metastases (39).

The usual criteria applicable for evaluating conventional chemotherapy drugs may fail to detect the efficacy of drugs targeted against RTKs. Inhibitors of RTKs stabilize the tumor in terms of cell proliferation, normal cell loss via apoptosis, and prevent cell migration, invasion and metastases. These drugs are likely to increase the time required for tumor progression, and may inhibit or attenuate the aggressiveness of the disease but may not initially result in measurable tumor regression. Therefore, specially designed trials are needed to evaluate the usefulness of drugs designed for RTK inhibi-

Table 2. Inhibitors of Receptor Tyrosine Kinases

Inhibitor	Tyrosine kinase target	Clinical trial	Source
Genistein	EGF-R, v-src		
Herbimycin A	EGF-R		
HNMPA-(AM)3	IR-K		
Lavendustin A	IR-K		
Tyrphostin 23	IR-K, EFG-R		
PP1	v-src		Pfizer
PP2	v-src		Pfizer
ZD 1839 (Iressa™)	EGF-R	Phase I (NSCLC, breast, colorectal, ovarian, renal, esophageal, pancreatic, and mesothelioma solid tumors)	Zeneca
CP-358774	EGF-R		Pfizer
CGP 59326	EGF-R		Novartis
PD 166285	EGF-R, PDGF-R, v-src		Parke-Davis
CGP 57148	PDGF-R		Novartis
SU101 (Leflunomide)	PDGF-R	Phase III (glioblastoma multiform) and Phase II (prostate, lung and ovarian cancer)	Sugen
SU 5416	Flk-1/KDR	Phase II (AIDS-related Kaposi sarcoma)	Sugen
STI 571	abl	Phase I (Philadelphia-chromosome-positive leukemia)	Novartis
ZD4190	VEGF-R		Astra Zeneca

tion. Perhaps drugs will act synergistically with the currently utilized chemotherapeutic agents. Inhibitors of RTKs are less likely to have adverse systemic toxicity since they are cytostatic and not cytocidal. They are likely to delay tumor progression by inhibiting cell cycle transit allowing other therapeutic agents additional time to cause tumor regression.

An example of cancer arising from a defective tyrosine kinase is a class of ALK positive lymphomas referred to as "ALKomas" which display inappropriate expression of a neural-specific tyrosine kinase, anaplastic lymphoma kinase (ALK) (40). Many solid tumors overexpress epidermal growth factor receptor (41). Iressa (ZD1839) is an orally active selective EGF-R inhibitor. This compound disrupts signaling involved in cancer cell proliferation, cell survival and tumor growth support by the host (42). The clinical efficacy of this agent shows that it is well tolerated by patients undergoing Phase I/II clinical trials (43,44). The compound has shown promising cytotoxicity towards several cancer cell lines (43).

Many growth factors and cytokines regulate cellular functions via the Janus kinase (JAK) signal transducers and activators of transcription (STAT). Membrane-associated JAK tyrosine kinases are activated upon ligand binding to an RTK. This preferentially recruits dormant cytoplasmic transcription factors (STATs) which are subsequently activated by phosphorylation. The phosphorylated STATS migrate to the nucleus and activate transcription of the target gene (45,46). Cells derived from rat and human cancers have constitutively activated Stat3, and the malignant potential of cancer has been associated with Stat3 activation (47,48). The JAK inhibitor AG940 prevents Stat3 activation and suppresses the growth of human prostate cancer cells (48).

DNA DEPENDENT PROTEIN KINASES

DNA-dependent protein kinase (DNA-PK) is involved in the repair of double-strand breaks in mammalian cells. This enzyme requires ends of double stranded DNA or transitions from single stranded to double stranded DNA in order to act as a serine/threonine kinase (49–54). Cells with defective or deficient DNA-PK activity are unable to repair radiation induced DNA double strand breaks and consequently very sensitive to the lethal effects of ionizing radiation (50–53). DNA-PK dependent repair of DNA double strand break involves DNA ligase IV and XRCC4 (53–55). Inhibition of DNA-PK has the potential to increase the efficacy of antitumor treatment with radiation or chemotherapeutic agents.

CELL CYCLE REGULATION BY CYCLIN DEPENDENT KINASES

Progression through the cell cycle is controlled in part by a series of regulatory molecules called cyclins and the cyclin-dependent kinases (CDK) which they activate. In addition to the cyclins, CDK activity is also regulated by phosphorylation and dephosphorylation. Cellular inhibitors of CDKs also play a major role in cell cycle progression (56). Alterations in the expression, function, and structure of cyclin and CDK are encountered in the cancer phenotype. Therefore CDKs may be important targets for new cancer therapeutic agents. Cell cycle perturbations occur in tumors and tumor cells treated with ionizing radiation and or chemotherapeutic agents. Whether or not the DNA damage caused in cells leads to cell death depends on normal cell cycle control mechanisms that are in place. Often chemotherapy resistant cells tend to escape apoptosis. Under certain circumstances, inappropriate CDK activation may even promote apoptosis by encouraging the progression of the cell cycle under unfavorable conditions, *i.e.,* attempting mitosis while DNA damage is largely unrepaired.

INHIBITION OF CDKs TO INDUCE APOPTOSIS IN CANCER CELLS

Purines and purine analogs act as CDK inhibitors. Flavopiridol (L86-8,275) is a flavonoid that causes 50% growth inhibition of tumor cells at 60 nM (57). It also inhibits EGFR and protein kinase A (IC_{50} about 100 μM) (57). Flavopiridel induces apoptosis and inhibits lymphoid, myeloid, colon, and prostate cancer cells grown *in vivo* as tumor xenografts in nude mice. At the molecular level, flavopiridol affects CDK function and arrests cells in the G_2/M and G_1/S border. Both cycling and non-cycling cells are killed by flavopiridol. At concentrations above 1 micromolar, flavopiridol loses its selectivity and starts inhibiting other kinases (e.g., 6 μM is the IC_{50} for protein kinase C) (57). Staurosporine and its derivative, UCN-01, in addition to inhibiting protein kinase C, inhibit cyclin B/CDK (IC_{50} 3 to 6 nM). Staurosporine is toxic, but its derivative 7-hydroxystaurosporine (UCN-01) has antitumor properties and is in clinical trials (58). UCN-01 affects the phosphorylation of CDKs and alters the cell cycle checkpoint functioning. These compounds illustrate that multiple intracellular targets may be affected as the concentration of an inhibitor is increased within cells.

TISSUE SPECIFICITY AS A COMPONENT OF IDENTIFYING THE THERAPEUTIC TARGET

Tamoxifen, a protein kinase C inhibitor with antiestrogen activity, is currently a standard treatment for hormone-dependent breast cancer. The use of this compound may increase the risk of developing cancer in other tissues such as the endometrium (59). Raloxifene, a related compound, has been shown to protect against osteoporosis (59). The tissue specificity of inhibitors must be considered when identifying therapeutic targets.

MITOGEN ACTIVATED KINASE (MAP KINASES) IN CARCINOGENESIS

Signal transduction to the nucleus in response to extracellular stimulus by a growth factor involves the mitogen activated protein (MAP) kinases. MAP kinases are a family of protein serine threonine kinases which mediate signal transduction from extracellular receptors or heat shock, or UV radiation (some receptors are tyrosine kinase receptors) (60,61). These kinases, in concert with other signal transduction pathways can network to differentially alter the phosphorylation of transcription factors. Cell proliferation and differentiation in normal cells are under the regulation and control of multiple MAP kinase cascades. Aberrant and deregulated functioning of MAP kinases can initiate and support carcinogenesis (62,63). Insulin and IGF-1 also activate a mitogenic MAP kinase pathway that may be important in

acquired insulin resistance occuring in type 2 diabetes (64).

PHOSPHATIDYLINOSITOL 3-KINASE, PKB/AKT AND CELL SURVIVAL

Many cancers become refractory to chemotherapy by developing a survival strategy involving the constitutive activation of the phosphatidylinositol 3-kinase-protein kinase B/Akt signaling cascade. This survival signaling pathway thus becomes an important target for the development of specific inhibitors that would block its function (65–68). PI-3 kinase/Akt signaling is equally important in diabetes (69). The pathway activated by RTKs subsequently regulates glycogen synthase kinase 3 (GSK3) and glucose uptake. Since Akt has decreased activity in type 2 diabetes, it provides a therapeutic target (69).

KINASE INHIBITORS AS TOOLS FOR STUDYING CELLULAR SIGNALING

Protein kinase inhibitors provide much of our knowledge about regulation and coordination of physiological functions. Endogenous peptide inhibitors occur *in vivo* (70). A pseudosubstrate sequence within PKC acts to inhibit the kinase in the absence of its lipid activator (71). A PKC inhibitor such as chelerythrine acts on the catalytic domain to block substrate interaction, while calphostin C acts on the regulatory domain to mimic the pseudosubstrate sequence and block ATPase activity, or by inhibiting cofactor binding. The ability to inhibit specific PKC isozymes is limited. The most specific inhibitors appear to be directed toward the conventional PKCs (regulated by phospholipids, calcium, and diacylglycerol) with at least two inhibitors of PKCβII identified (72,73). Most PKC inhibitors, including those for PKCβII, inhibit insulin-induced glucose uptake (73,78). The importance of PKC activation for insulin action has been the topic of numerous studies. Multiple PKC isozymes appear to be involved in regulating glucose uptake (78) and insulin resistance mediated by the insulin receptor (79).

The caveat for evaluating the specific function of a kinase using inhibitors lies in the non-specific actions of some compounds and their ability to inhibit a number of different protein kinases or, at higher concentrations, similar isozymes. The cellular uptake, half-life, diffusion, or multiple intracellular receptors are also considerations when interpreting inhibitor effects on metabolic and mitogenic function.

Activated kinases can have multiple substrates that are then trafficked to subcellular locations via phosphorylation/dephosphorylation signals. Nuclear targets of activated kinases are thought to be transcriptional activation factors. Another mechanism of activating transcription factors that are dormant in the cytoplasm is their translocation into the nucleus upon phosphorylation. This mechanism of signal transduction is observed in the case of NF-κB proteins (74). NF-κB complexes are inactive due to complexing with IκB inhibitors, but upon phosphorylation of the regulatory IκB by PKC and PKA, free NF-κB complexes are dissociated and are then translocated to the nucleus (74).

OTHER MODES OF REGULATING PROTEIN KINASES

Although some protein kinases have, to date, no known system of physiological regulation, many are activated or in-

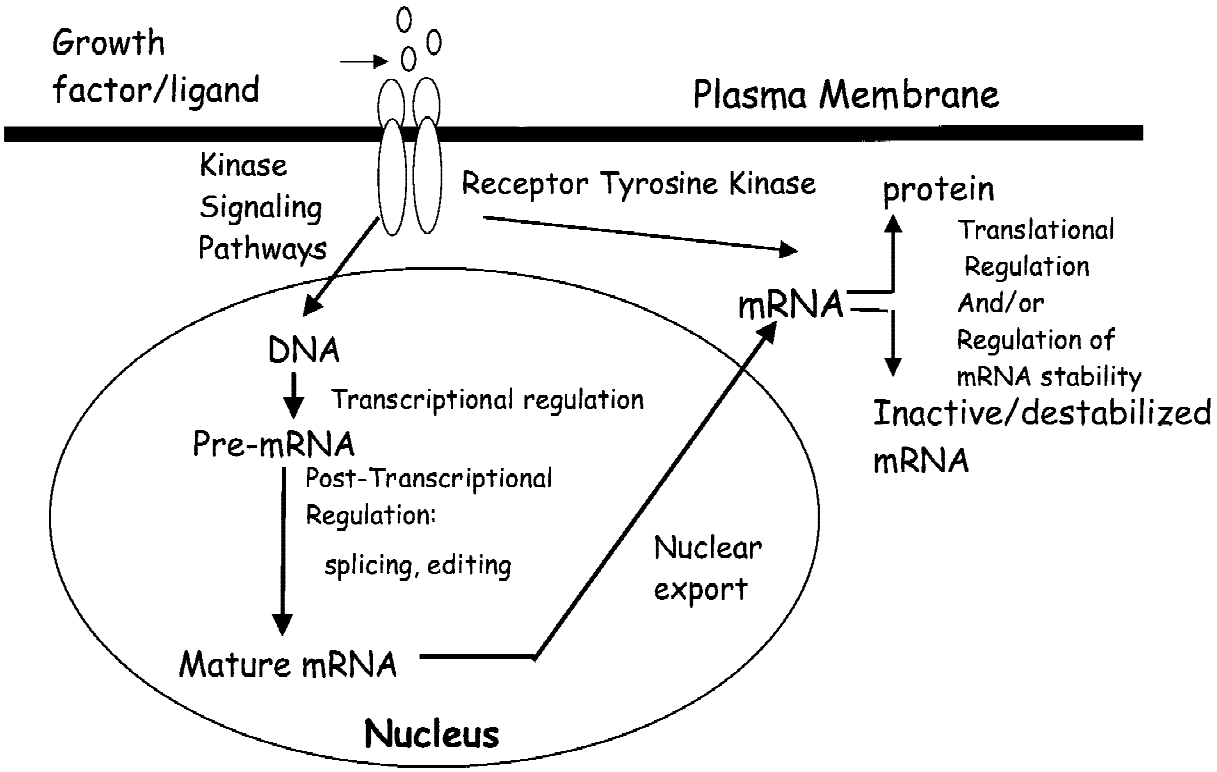

Fig. 3. Regulatory control points in gene expression by growth factors. Protein kinase signaling pathways activated by protein tyrosine kinases are known to regulate RNA transcription, post-transcriptional processing of pre-mRNA, mRNA stability, and protein phosphorylation.

activated by autophosphorylation or phosphorylation by upstream protein kinases. The regulation of protein kinases also occurs transcriptionally, post-transcriptionally, and post-translationally (Figure 3). The mechanism of post-transcriptional regulation is alternative splicing of precursor mRNA (75). Protein kinase C-βI and -βII are two isoforms of a single PKCβ gene derived from differences in the splicing of the exon encoding the C-terminal 50–52 amino acids. Splicing can be regulated by a kinase cascade in response to peptide hormones such as insulin and IGF-1 (75). PKCβI and βII have different specificities for phosphorylating members of the mitogen activated protein (MAP) kinase family, for glycogen synthase 3β, for nuclear transcription factors such as TLS/Fus, and for other nuclear kinases (76–78). By inhibiting the post-transcriptional alternative splicing of PKCβII mRNA, PKCβII-dependent processes are inhibited.

The stability of mRNA encoding the PKC isozymes is also apparently regulated by kinase cascades. Destabilization of PKCδ mRNA by phorbol esters is one example (80). The destabilization of PKCβII mRNA by glucose is another case in which stability is modulated by protein kinases (81). Thus, regulation of PKCβII expression by insulin via alternative splicing of pre-mRNA and glucose via destabilization of mRNA, suggests that post-transcriptional processing may be a likely target for altering kinase levels. The development of antisense oligonucleotides to inhibit the expression of various protein kinases has been successful. Antisense oligonucleotides are short lengths of synthetically manufactured, chemically modified DNA or RNA designed to specifically interact with mRNA transcripts encoding target proteins. The interaction of the antisense moiety with mRNA inhibits protein translation and, in some cases, post-transcriptional processing (e.g., alternative splicing and stability) of mRNA. Antisense oligonuclotides have been developed to alter alternative splicing of BclX long to short mRNA forms and for inhibiting the translation of PKCα and PKC ζ (82).

PROTEIN KINASE INHIBITORS IN CARDIOVASCULAR DISEASE AND VASCULAR COMPLICATIONS IN DIABETES MELLITUS

Protein kinase C isoforms have been implicated in cellular changes observed in the vascular complications of diabetes. Hyperglycemia is associated with increased levels of PKCα and β isoforms in renal glomeruli of diabetic rats (72). Oral administration of a PKCβ inhibitor prevented the increased mRNA expression of TGF-βI and extracellular matrix component genes (72). Administration of the specific PKCβ inhibitor (LY333531) also normalized levels of cytokines, caldesmon and hemodynamics of retinal and renal blood flow (72). Overexpression of the PKCβ isoform in the myocardium resulted in cardiac hypertrophy and failure (72). The use of LY333531 to prevent adverse effects of cardiac PKCβ overexpression in diabetic subjects is under investigation (72). The compound is also in Phase II/III clinical trials for diabetic retinopathy and diabetic macular edema indicating that it may be pharmacodynamically active (83).

CONCLUSIONS

Our original understanding of kinases and their roles in cellular metabolism are based on work by Krebs, Graves and Fisher (84), more recently, information provided from investigations on such diverse species as *D. melanogaster, S. cerevisiae, D. discoideum, and C. elegans* has identified new kinase genes and allowed cloning of their mammalian counterparts (1,3). The further use of peptide libraries, protein-RNA, protein-DNA, and protein-protein interaction systems will advance our understanding of kinase specificity and how kinases are regulated by protein interaction, and will provide additional molecular possibilities for drug intervention.

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