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Protein Kinase C and Growth Regulation in Malignant Gliomas

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To the Editor: The presence of amplified growth factor systems in many tumor models, including malignant gliomas, raises the question of how such external signals are transduced into a transformed phenotype (increased proliferation, invasion, lack of contact inhibition, angiogenic capacity, etc.). In this regard, intracellular signal transduction systems are likely to play a key role. Such amplified external signals, by way of transduction systems, may alter genomic expression to effect a change of the expression of structural or regulatory genes to produce the malignant phenotype. Furthermore, the complexity and multiplicity of the growth factor systems place therapeutic impetus upon determining events that follow growth factor/receptor binding. Determination of postbinding events may reveal common intracellular transduction mechanisms activated by these different signals.

One of these candidate signal transduction systems is the protein kinase C (PKC) system. This multifunctional and ubiquitous enzyme system family has recently been a focus of interest in the study of human gliomas. PKC is a phospholipid-dependent kinase that is very active in central nervous system tissue (Fig. 1). It has been shown to be ubiquitous in distribution, with a striking expression in brain. Though 12 subspecies (isozymes) of PKC have been identified, differences in regulation or substrate specificity of the isoforms have not been defined. As these isoforms have preferential expression among different cell types, it is likely that isozyme-specific function in certain cell types will be identified.

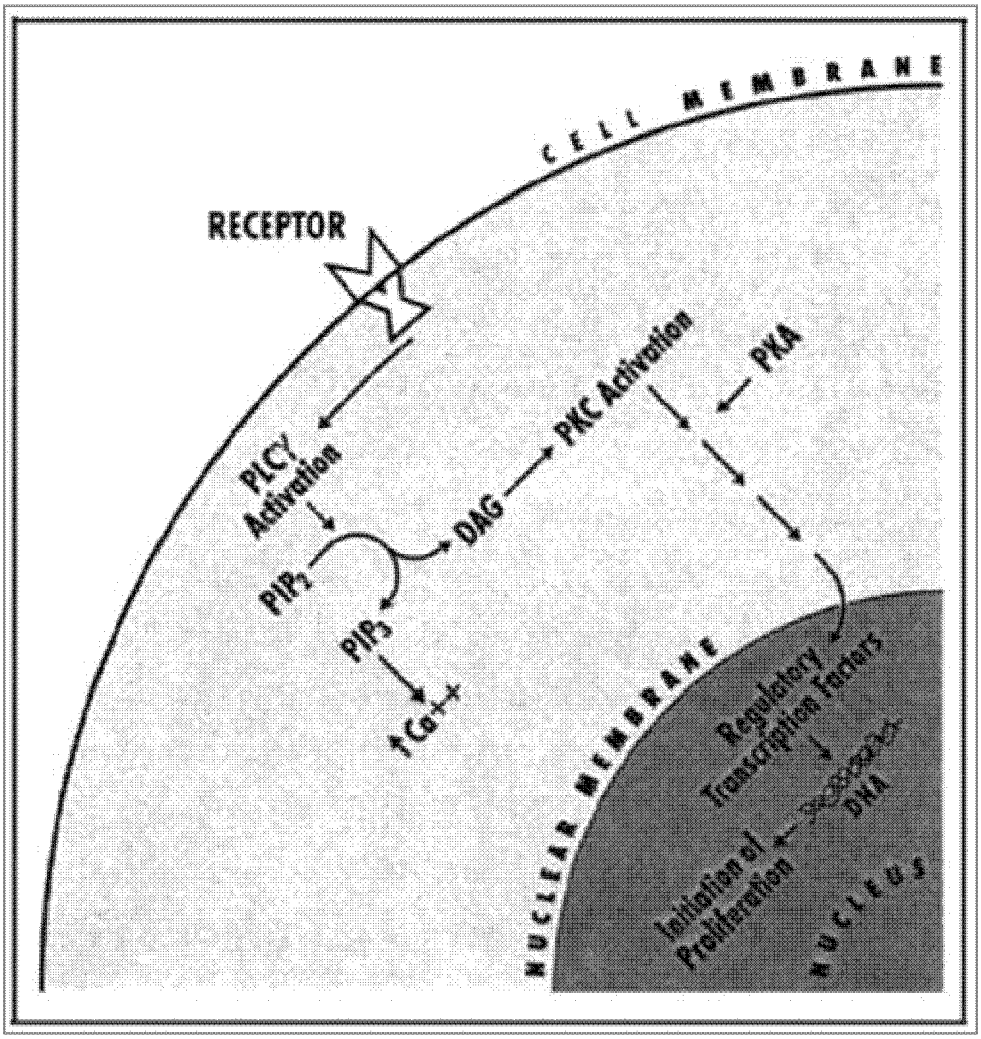

Figure 1. The role of PKC in signal transduction. After activation of an appropriate growth factor-stimulated cell surface receptor, membrane-associated inositol phospholipids are hydrolyzed, producing diacylglycerol (*DAG*) and inositol phosphates. It is thought that the primary effect of the diacylglycerol liberated is the activation of PKC, which, in turn, activates a sequential series of intracellular kinases, resulting in potential alteration of gene expression through phosphorylation of regulatory transcription factors in the nucleus. This is a multi-step process, simplified in this diagram, which involves several intracellular kinases, such as *Raf-1*, *Ras*, and *MAP* (mitogen-activated protein) kinases (not shown). The end result of alteration of these transcription factors may be a signal to undergo cellular division (proliferation). Note that there is interaction between signal transduction systems (protein kinase A [PKA] illustrated here) resulting in so-called "cross-talk" and some mutual interdependence of activity. PLC, phospholipase C; PIP, phosphatidyl inositol phosphate; DNA, deoxyribonucleic acid.

Mitogenic signaling through PKC—Previous work has demonstrated exquisite sensitivity of the growth rates of malignant gliomas to modulators of PKC in vitro. Malignant gliomas express very high PKC activity when compared with nontransformed glial cells (approximately 1000-fold increase), in both human and rat systems. The high

activity strongly correlates with the proliferation rates of these tumors in vitro. Moreover, the identified glioma mitogens, fibroblast growth factor and epidermal growth factor, and serum mitogens induce PKC activity, which correlates with the increase in [^{3}H]thymidine uptake produced by these mitogens. Altering enzyme activity by down-regulation or direct inhibition reduces the tumor growth rate significantly in vitro. The increased PKC activity thus represents a biochemical and functional marker of malignancy in these astroglial tumors. In addition to its role in the regulation of proliferation in these tumor cells, recent work has demonstrated a potential important role of PKC in the regulation of glioma cell invasion in vitro.

Moreover, it appears that the PKC system is uniquely dysregulated in these tumors. For example, the isozyme profile in the glioma cell is aberrant, in that Western blots performed on several established and low-passage malignant glioma cell lines indicate no detectable signal from the ß-isoform, which is present in nontransformed glia. The precise role of each of these isoforms is an area of ongoing research effort to define specific isoform(s) that hold potential for growth modulation in these cells.

Signal transduction systems as a chemotherapeutic target—From the discussion above, intracellular signal transduction systems offer a natural "choke point" for the regulation of cell growth from a divergent number of external signals. However, when considering any role for antisignal transduction therapy, the natural question arises as to how one can utilize chemotherapy directed at such intracellular targets when these systems play a ubiquitous role in nontransformed cells in the central nervous system and elsewhere. The answer may lie in some recent unexpected laboratory and clinical observations.

Tamoxifen, in a property distinct from its well-established estrogen receptor blockade, also inhibits PKC in the micromolar concentration range in glioma cells. Tamoxifen has previously been shown to inhibit PKC in other cell types and to decrease the proliferation rates of established glioma cell lines in vitro. It inhibits approximately 25 to 30% of low-passage glioma specimens in vitro. A study administering low-dosage tamoxifen (antiestrogen dosage of 40 mg/d) to patients with recurrent malignant gliomas, while failing to demonstrate statistically increased survival, documented isolated cases with apparent response to the treatment. However, administration of high-dose tamoxifen (at least 200 mg/d, dosages chosen to produce tissue levels in the low-micromolar range and shown necessary to inhibit PKC) has been recently reported to induce stabilization or tumor regression in up to 40% of patients with recurrent malignant gliomas following standard external beam radiation therapy with or without chemotherapy. Though other pleiotropic effects of tamoxifen may also be responsible for this antitumor effect, PKC inhibition is a likely candidate. Moreover, an unexpected finding in many of these patients was actual tumor regression; from laboratory work prior to the initiation of these trials, we expected only stabilization (i.e., growth arrest or cytostasis).

One mechanism that may explain the clinical finding of tamoxifen-induced tumor

regression is revealed by some recent observations from our laboratory, which demonstrate that glioma cells undergo apoptosis (or programmed cell death) in vitro in response to tamoxifen, staurosporine, and hypericin, all of which inhibit PKC activity (2,3). A salient feature of apoptotic cell death is the degradation of genomic deoxyribonucleic acid to oligonucleosomal fragments (fragments of deoxyribonucleic acid of short length), presumably by a consequence of activation of one or more of a family of endonucleases. The finding that PKC inhibitors induce apoptosis in glioma cells is an exciting new development in light of the many recent studies that have implicated oncogenes and tumor suppressor genes as important regulators of cell survival or death (reviewed in Refs. 1 and 6). These studies suggest that the pathways for cell proliferation and cell death may be tightly coupled, with cells displaying dysregulated proliferation being particularly prone to undergoing apoptosis. Pharmacological intervention directed at critical signal transduction factors, such as PKC, may perturb the balance between cell proliferation and apoptosis resulting in tumor cell death (5).

What role might elevated PKC activity play in the regulation of apoptosis in gliomas? These tumors, like many others, also overexpress the c-*myc* protooncogene, which encodes a transcription factor that promotes cell proliferation by modulating the expression of growth-related target genes (reviewed in Ref. 1). Elevated levels of c-*myc* correlate with both increased proliferative rate and enhanced sensitivity to apoptosis (4). The apparent paradox that mitogenic protooncogenes participate in both tumorigenesis and tumor cell apoptosis can be reconciled by the finding that tumors have developed mechanisms to suppress the apoptotic response. The model presented in Figure 2 proposes a central role for PKC in regulating pathways controlling glioma proliferation and survival. In response to mitogens, PKC activity is elevated, which provides dual signals for driving cell proliferation while suppressing apoptosis. As illustrated in the figure, the apoptotic signal delivered by c-*myc* can be blocked by either deregulated expression of the *bcl*-2 protooncogene or loss of normal p53 tumor suppressor activity. Elevated PKC activity in gliomas may function analogously as a negative regulator of apoptosis. Inhibition of PKC activity by tamoxifen would release the block on the apoptosis pathway leading to increased tumor cell death. The ability of PKC inhibitors to induce apoptosis in gliomas may also depend on the presence or absence of other negative regulators of apoptosis, such as *bcl*-2 and mutated p53. Signalling through the p55 tumor necrosis factor receptor or the Fas antigen (a member of the tumor necrosis factor/nerve growth factor receptor family) can also induce apoptosis in glioma cells. The tumor necrosis factor-[alpha] action has been demonstrated to be mediated, in part, through PKC in other cell types.

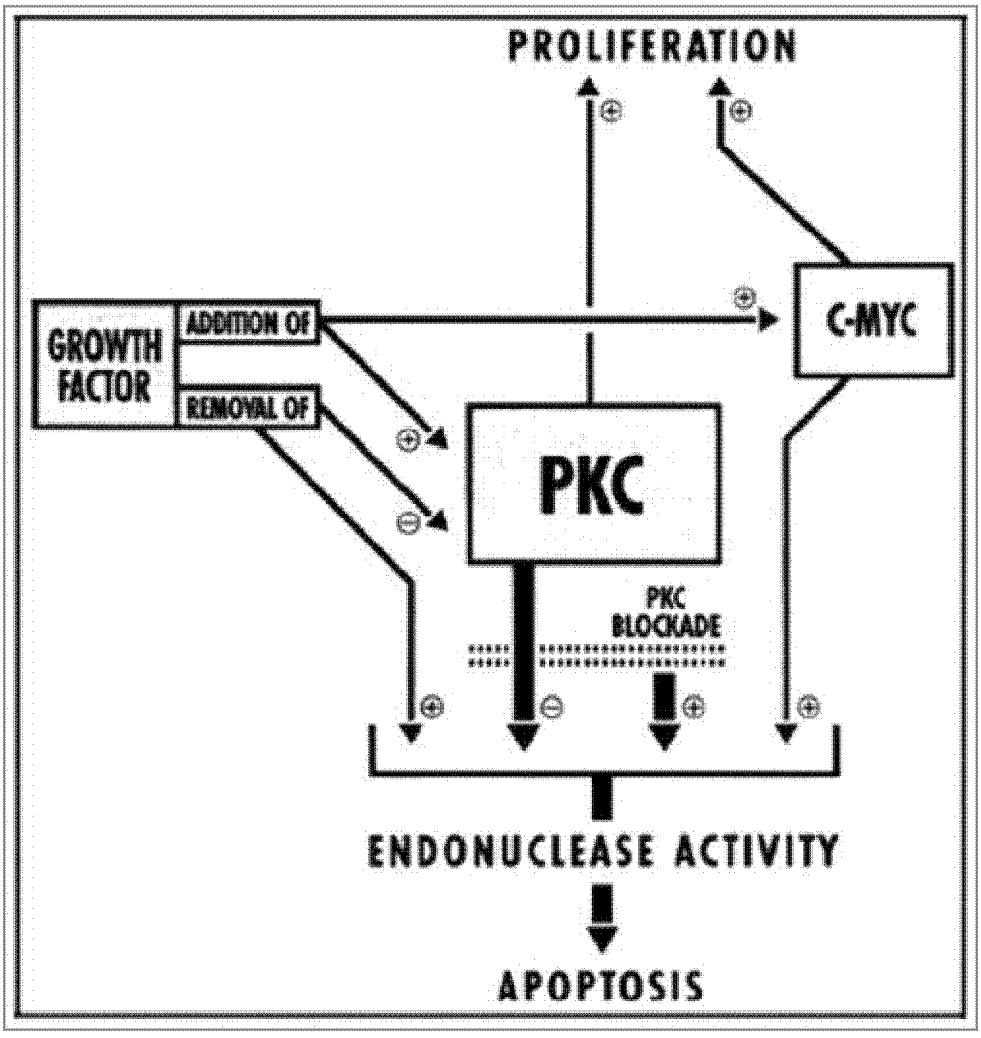

Figure 2. A postulated role for PKC in the delicate balance between proliferation and apoptosis in transformed cells. Model to explain how PKC may transduce signals that determine whether glioma cells proliferate or undergo apoptosis. In response to mitogens or as a consequence of genetically dysregulated growth factor signaling pathways, gliomas have very high PKC activity. In conjunction with elevated or dysregulated expression of mitogenic proto-oncogenes, such as c-*myc*, gliomas are driven to proliferate. Elevated PKC also functions to block activation of the signaling pathway leading to apoptosis, which can be triggered by overexpression of the same proto-oncogene(s), such as c-*myc*, required for enhanced proliferation. Inhibition of PKC activity in gliomas by drugs such as tamoxifen, staurosporine, or hypericin relieves the block imposed on the pathway for apoptosis resulting in increased tumor cell death.

If such an apoptosis pathway is initiated in vivo following the administration of PKC inhibitors, the important clinical implication is that this therapy may be cytocidal (i.e., kill cells) and not merely cytostatic (inhibit growth). In this regard, a percentage of the patients in our personal study have achieved complete radiographic remission (on magnetic resonance imaging and positron emission tomography studies) following extended high-dose tamoxifen therapy; tamoxifen has been discontinued in these patients, and extended follow-up will the necessary to determine the true tumor control

from this therapy.

Finally, if PKC is the true intracellular target for the antiglioma effects of tamoxifen, it emphasizes the importance of attempting to identify clinically applicable agents with increased efficacy and/or specificity. Tamoxifen may, at best, be considered only a moderately potent agent, as studies have illustrated that other kinase inhibitors, such as staurosporine, possess some 1000-fold higher activity against PKC and glioma growth. Another agent, hypericin (3), which has been clinically used as an antidepressant, crosses the blood brain barrier effectively and demonstrates superior growth inhibitory and apoptosis induction properties to tamoxifen in vitro. Such studies suggest that this agent may be suitable for future chemotherapeutic trials in humans with malignant gliomas, and ongoing work by several groups is focused on identification of other kinase inhibitors with increased antitumor activity.

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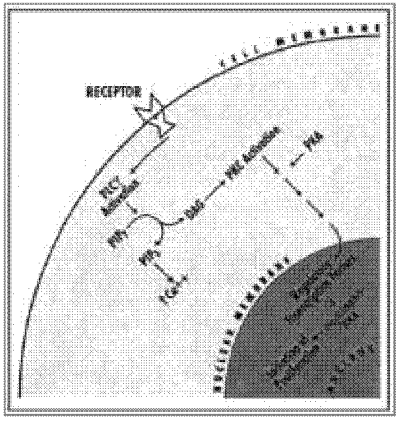

Figure 1

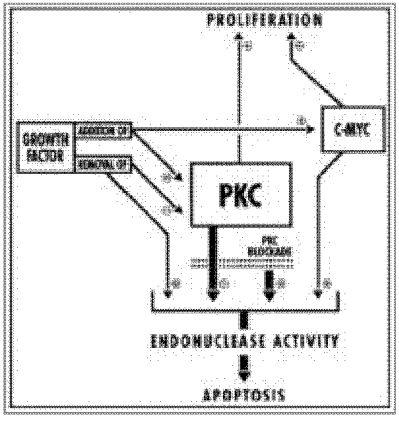

Figure 2

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