

Protein Kinase C and Growth Regulation of Malignant Gliomas

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ABSTRACT: This article reviews the role of the signal transduction enzyme protein kinase C in the regulation of growth of malignant gliomas, and describes how targeting this enzyme clinically can provide a novel approach to glioma therapy.

RÉSUMÉ: Protéine kinase C et régulation de la croissance des gliomes malins. Nous revoyons le rôle de l'enzyme de transduction du signal, la protéine kinase C, dans la régulation de la croissance des gliomes malins et nous décrivons comment le ciblage de cet enzyme peut fournir une approche nouvelle dans le traitement du gliome.

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Over 50% of tumors that originate within the central nervous system are gliomas.¹ These glial neoplasms are classified as either pilocytic astrocytoma (Grade I), low grade astrocytoma (Grade II), anaplastic astrocytoma (Grade III), or glioblastoma multiforme (Grade IV).² While complete surgical resection of pilocytic astrocytoma can result in cure, incompletely resected tumours, despite adjuvant therapy, invariably recur and often at a more malignant grade. High grade gliomas have a very poor prognosis with only 10% of patients surviving beyond 2 years.¹ This statistic has not improved significantly in the last 15 years despite improved imaging, advances in neurosurgical technique, and new forms of adjuvant therapy including brachytherapy, immunotherapy, and chemotherapeutic agents. A better understanding of glioma genesis and growth characteristics is therefore required in order to improve treatment strategies for patients with this common brain neoplasm.

Cytogenetic studies have revealed many chromosomal changes in malignant astrocytomas, including increased numbers of chromosome 7, decreased numbers of chromosome 10, and deletions in chromosomes 9p and 17p.³⁻⁶ These chromosomal changes have resulted in specific molecular alterations (amplifications, deletions, and mutations) in several tumor suppressor genes, protooncogene-coded growth factors, or growth factor receptors.⁷⁻¹⁴ Whereas chromosome 17p loss and p53 inactivating mutations are probably involved early in the pathway of tumorigenesis of many astrocytomas, since these abnormalities can be detected in low grade gliomas, epidermal growth factor receptor (EGFR) amplification and chromosome 10 loss are seen predominantly in only high grade gliomas.¹⁵⁻¹⁹ p53 alterations are observed in up to 70% of cases of human gliomas while amplification or gene arrangement for N-myc, c-myc and N-ras are rare in gliomas.¹⁹

Since many oncogene products phosphorylate the tyrosine residues on proteins, much attention has been given to the role of tyrosine kinase in tumorigenesis. However, in recent years there has been an increasing recognition for the role of protein kinase C (PKC), an important signal transduction enzyme that

conveys signals generated by ligand-receptor interaction at the cell surface to the nucleus, as a critical regulator of cell growth and transformation.²⁰⁻²² This article will describe the evidence that implicate PKC as a key regulator of the growth of malignant gliomas.

PROTEIN KINASE C BIOCHEMISTRY

Protein kinase C (PKC) is a family of serine/threonine kinases that was first characterized on the basis of its activation *in vitro* by Ca²⁺, phospholipid and diacylglycerol (DAG).²³ At least 11 isoforms are currently known to exist²³⁻²⁵ and these can be subdivided into 3 groups based on their mode of activation (Figure 1). The first group of isoforms consists of the 4 classical PKCs (cPKC): α , β_1 , β_2 and γ , and are dependent on Ca²⁺, phosphatidylserine and DAG for activation. The second group of isoforms consist of 5 novel PKCs (nPKC): δ , ϵ , η (L), θ and μ , and are dependent on phosphatidylserine and DAG, but not Ca²⁺, for stimulation; it is thought by some that PKC μ should be in a class of its own since this is the only PKC isoform which has a transmembrane domain.^{26,27} The third group of isoforms, ζ and ι (λ is the mouse homolog of human ι), are atypical PKCs (aPKC) which are dependent on phosphatidylserine, but not Ca²⁺ or DAG for activation.

The classical PKC's possess 4 conserved (C1 to C4) and 5 variable (V1 to V5) regions (Figure 1). The C1 region contains a tandem repeat of 2 cysteine-rich zinc finger structures; the first binds DAG and phorbol esters (PE) while the second structure is thought to be the binding site for phospholipids. The C1 region

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contains a stretch of amino acid sequences known as the pseudosubstrate sequence that resembles a PKC substrate but contains alanine and not any serine or threonine residues that can be phosphorylated. This pseudosubstrate region, by binding to the substrate docking region of the C4 domain (the V3 hinge region allows for the folding of the PKC enzyme), is important in keeping PKC in its inactive form. The C2 region contains the calcium binding site. Besides binding calcium, the C2 region is a likely binding site for RACKs (receptor activated C-kinase; 3 putative RACKs of MW 28, 30, and 33 kDa have been identified) which are "intracellular PKC receptors" that influence the activation-induced translocation of PKC from the cytosol to the particulate fractions.²⁸ The C3 region contains a conserved ATP-binding domain. C4 contains the substrate docking domain. The C1 and C2 regions (together with V1 and V2) comprise the regulatory domain of PKC while the C3 and C4 regions together with V4 and V5 form the kinase domain.

The novel PKC isoforms (δ , ϵ , η (L), θ and μ) lack the calcium-binding C2 region while the atypical PKC's, aside from the lack of the C2 region, have only one cysteine-rich zinc finger-motif leading to a loss of responsiveness to DAG and its pharmacological ligand, phorbol esters.

PKC is generally thought to be activated in the cell by signal transduction cascades that produce DAG, such as through certain tyrosine kinase receptors that can stimulate phospholipase C γ .²³ It is presently thought that upon appropriate ligand-receptor interaction at the cell surface, and through the mediation of G proteins signal transducers, phospholipase C γ catalyses the

breakdown of PIP₂ (phosphatidylinositol 4,5-bisphosphate) into IP₃ (inositol 1,4,5-triphosphate) and DAG.²⁹ IP₃ mobilises Ca²⁺ from internal stores and the binding of Ca²⁺ to the C2 region, with the help of RACKs through unknown mechanisms, presumably results in translocation of PKC from the cytosol to the cell membrane. At the cell membrane, DAG generated by PIP₂ turnover, and phosphatidylserine, present constitutively in the cell membrane, bind to the C1 region. The presence of each of the co-factors increases the affinity of one another for binding to the PKC molecule. It has been proposed that the active complex is a monomeric PKC, 4 molecules of phosphatidylserine, 1 molecule of DAG, and at least 1 Ca²⁺ ion.³⁰

In the absence of activating co-factors (Ca²⁺, phospholipid, or DAG/PE), the pseudosubstrate motif at the C1 domain of the regulatory region interacts with the substrate docking site of the C4 domain of the catalytic region and thus prevents access of substrates to the catalytic site.³¹ Cofactor binding reduces the affinity of pseudosubstrate site-catalytic site interaction and thus exposes the substrate docking pocket to PKC substrates.

The mechanism by which PKC is inactivated remains complicated, and may involve the decreased availability of co-factors, or the degradation of PKC enzyme in a down-regulation phenomenon.²³ It was originally thought that the existence of DAG is transient and that phorbol esters are potent PKC stimulators because of their relative resistance to degradation. It is now known that DAG can be generated from different sources and depending on the source, the existence of DAG can be prolonged leading to sustained PKC activation.²³ Thus, upon cell stimulation, the early peak of DAG, formed from PIP₂ and

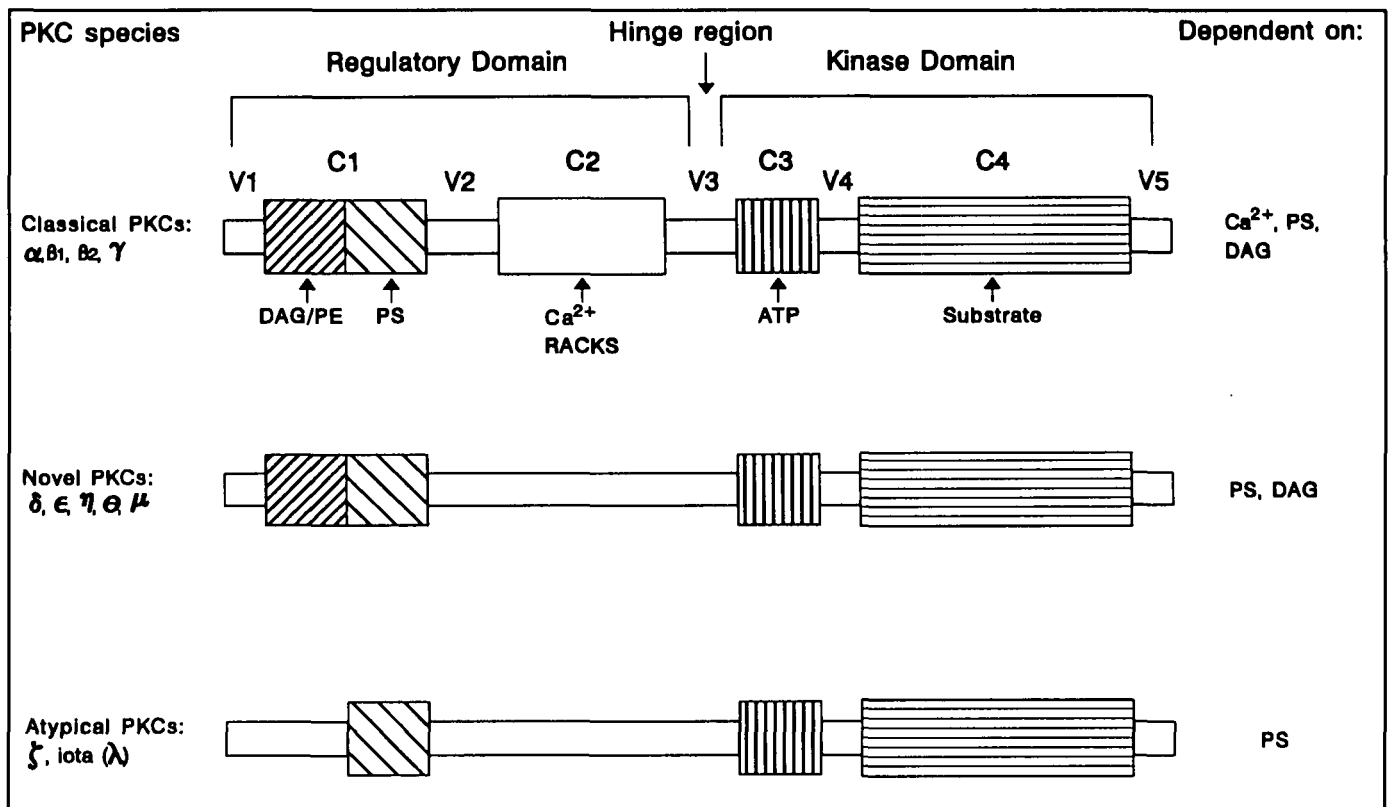


Figure 1: Structure of PKC isozymes.

coinciding with the IP₃ and Ca²⁺ increases, is transient and reverts back to baseline within seconds, at most several minutes. At a relatively later phase of cellular responses, the formation of DAG has a slower onset but is sustained. This is more likely derived from the hydrolysis of major constituents of the phospholipid bilayer such as phosphatidyl choline (PC) by phospholipase D, yielding phosphatidic acid (PA) which is then dephosphorylated to DAG. PC is also hydrolysed by phospholipase A₂ to yield cis-unsaturated fatty acids and lysophosphatidylcholine, which are both enhancer molecules of PKC activation.

The histological distribution of PKC isoforms is tissue-, as well as cell type-specific. A consensus is that γ is brain-specific, and that α , δ , and ζ are universally distributed.³² A very good example of the distribution of PKC isoforms being cell type-specific is in the adult rat pancreas where the β , α , and δ cells express only PKC α , γ and ϵ respectively.³³ Another example of cell type-specific distribution is in the retina, where PKC ζ was exclusively associated with the inner segments of the photoreceptors, ϵ was present in bipolar cells, and δ in Muller cells and a subpopulation of ganglion cells.³⁴

PKC AND CELL GROWTH

The role of PKC in cell growth was first suggested following observations that the tumor promoting phorbol esters are pharmacological activators of PKC.³⁵ Many oncogene products such as the epidermal growth factor (EGF) receptor homolog neu/erb-B2 increase the levels of DAGs, endogenous ligands for PKC. It has been hypothesized that the persistent activation of PKC in ras transfected cells is one of the mechanisms that leads to malignancy.³⁶ In breast tumors and pituitary adenomas, increased PKC content and activity are found in the tumor tissue compared to normal tissue.^{37,38} In colon cells, progressive alterations of PKC enzyme activity have been associated with transformation into a more malignant phenotype.³⁹

Specific isoforms of PKC have been implicated in the metastatic potential of various types of tumor cells including leukemia cells, bladder carcinomas, and melanomas.⁴⁰⁻⁴² Cells transformed by a variety of oncogenes exhibit marked changes in their PKC isozyme profile in addition to altered rates of proliferation.⁴³⁻⁴⁵ Overexpression of PKC α increased growth in Swiss 3T3 cells⁴⁶ whereas overexpression of the same isoform decreased growth in melanocytes.⁴⁷ Overexpression of PKC β_1 can confer multiple growth abnormalities in rat fibroblasts;⁴⁸ on the other hand, PKC β may play a tumor suppressor role in colon cells⁴⁹ and melanocytes.⁵⁰ PKC ϵ has recently been reported to be itself an oncogene when overexpressed in R6 rat fibroblasts⁵¹ and in NIH 3T3 cells;⁵² however overexpression of PKC δ resulted in reduced growth in the same NIH 3T3 cells (Michak et al., 1993). These observations strongly suggest that the various isoforms of PKC play a critical role in carcinogenesis and that individual isoforms of PKC can play distinctive roles in the process of cell transformation even within the same cell type. The reason for these distinctive functions in different cell types is unclear. Dekker and Parker²⁵ have proposed that it is the differences in intrinsic substrate specificity of the PKC isoforms, the availability of particular substrates, and the colocalization of substrate and enzyme within the cell which are the major functional determinants.

PKC AND THE CNS

The high level of protein kinase C (PKC) expression in the brain suggests that this enzyme system plays an important role in the normal functioning of cells of the central nervous system.⁵³ PKC has been implicated in long term potentiation in neurons,⁵⁴ neurite extension,^{55,56} release of neurotransmitters (reviewed in 23), and process formation by oligodendrocytes.^{57,58} PKC also has a critical role in regulating astrocyte growth as suggested by the findings that phorbol esters can affect the differentiation,^{59,60} morphology,^{61,62} and proliferation rate⁶³⁻⁶⁵ of astrocytes in vitro. As well, we have found that there is a high degree of correlation between PKC activity and the proliferation rate of human and rodent astrocytes in culture; furthermore, the mitogenic effects of several growth factors on astrocytes⁶⁶ are abrogated by agents that inhibit PKC.⁶⁷

PKC AND GLIOMA

The apparent dependence on PKC of mitogenic signalling in astrocytes suggested to us that abnormalities of PKC may underlie the hyper-proliferative state of glioma cells, since these transformed cells usually arise from a glial precursor. In this regard, work from this laboratory has determined that malignant human glioma cells in vitro have a very high enzyme activity of PKC that correlates with their rapid growth rate.⁶⁸ In comparison with non-malignant adult human glia, the PKC enzyme activity of glioma cells was at least an order of magnitude higher. The high PKC enzyme activity appears to be inherent to glioma cells but not to non-glial tumor types (e.g., colorectal, cervical and bladder carcinoma) with equal or faster growth rates.⁶⁹ Within each glioma cell line of 5 studied, the PKC activity of each at each phase of growth (slow, log, and plateau) correlates with the respective rate of proliferation at that stage.⁶⁸ Furthermore, mitogens for glioma cells (EGF and serum) translocated PKC from the cytosol to the particulate fraction of glioma cells.⁷⁰ As well, in concordance with Pollack et al.,⁷¹ the proliferation rate of glioma cells in vitro can be reduced by over 90% by inhibitors (staurosporine, tamoxifen and CGP 41251) of PKC.^{69,70,95} Although these inhibitors are relatively non-selective for PKC, the IC₅₀ values for inhibition of PKC enzyme activity by these agents correspond to their respective IC₅₀ values for inhibition of glioma cell proliferation,^{69,95} supporting the contention that reduction of glioma cell proliferation is via a PKC-dependent mechanism.

The high PKC enzyme activity that is inherent to glioma cells when compared to non-malignant glia raises the issue of whether this is a result of overexpression of particular PKC isozymes. We have taken advantage of the capability of obtaining rat astrocytes in high purity (over 95%) and quantity from the neonatal brain to address whether there is differential expression of PKC isoforms between the rat C6 glioma cell line and non-neoplastic astrocytes, and whether a particular PKC isozyme may be overexpressed in C6 glioma cells to account for their high PKC enzyme activity and proliferation rate. We found the PKC profiles of rat astrocytes and C6 to be similar, with expression of α , β , δ , ϵ and ζ but not γ .⁷² Furthermore, when PKC enzyme activity was assayed, C6 cells were found to be at least an order of magnitude higher than astrocytes. This elevated activity was predominantly Ca²⁺-dependent (ie., classical PKC

isozymes), implicating PKC α , since C6 glioma cells have minimal amounts of β and undetectable quantities of γ . Indeed, Western immunoblots revealed an overabundance (of at least 10 fold and as high as 60 times) of PKC α in C6 cells compared to astrocytes (Figure 2).⁷² Finally, an antisense oligonucleotide directed against the site of initiation of PKC α was found to decrease PKC α protein and proliferation rate of rat⁷² or human glioma cells⁷³ in vitro.

It is presently unclear whether the overexpression of PKC α in C6 glioma cells is secondary to other genetic changes, or whether this aberration represents a more primary event; these will have to be determined. Furthermore, glioma cells also contain PKC δ , ϵ , and ζ and whether these isozymes affect other aspects of glioma biology remains unclear.

It is imperative to analyse tumor specimens in situ to address whether PKC, and in particular PKC α , is high in gliomas in vivo. Analyses using tissue homogenates would be difficult since other cell types also contain PKC; the best approaches would likely be immunohistochemistry or in situ hybridization for particular PKC isoforms on tissue sections where the malignant cells can histologically be clearly identified.

In recent years, there has also been increased interest in other laboratories on PKC in gliomas. Reifenberger et al.⁷⁴ observed the presence of PKC α and PKC β in glioma specimens while Todo et al.⁷⁵ found strong immunoreactivity for PKC α , but weak signals for β and γ , in glioblastoma cells in situ. Benzil et al.,⁷⁶ by Northern blot analyses, demonstrated that the expression of PKC α was high in well-differentiated astrocytomas, intermediate in anaplastic astrocytomas and low or non detectable in glioblastomas; PKC β transcript was not detected in any gliomas, while PKC γ transcript was present in one of four anaplastic astrocytomas. As aforementioned, a cautionary note is worthy of mention when analysing results obtained from homogenates of brain. These homogenates (whether for protein, DNA or RNA determinations) contain a variety of cell types and the results would reflect the relative contribution by neurons, non-malignant glia, and blood vessels. Indeed, the contaminat-

ing cell types and their respective contributions of PKC isoforms to tissue homogenates will vary vastly between low and high grade gliomas. In particular, low grade specimens would contain neuronal elements and non-transformed glia, while high grade specimens would contain less neurons or glial elements, but higher amounts of angiogenic blood vessels and areas of necrosis. In normal brain, or in vitro, PKC is generally higher in neurons than in other cell types.⁷⁷⁻⁷⁹

In vitro, Shimosawa et al.⁸⁰ found glioma cells to contain PKC α , but not β or γ , while Misra-Press et al.,⁸¹ studying the same cell line (A172), found PKC α and γ , but not PKC β expression; PKC γ has been previously thought to be neuron-specific and not to be found in glial cells.⁷⁹ Recently, two separate reports demonstrate the presence of α , γ , ϵ and ζ isoforms, and the absence of β and δ in human glioma cell lines;^{82,83} however another report observed the presence of α , δ and ζ , and the absence of β , γ and ϵ .⁸⁴ Many factors may contribute to the observed discrepancies in PKC isoform expression including the specificity of the antibodies used, the particular cell line in question, and other factors; we would need to clarify whether particular PKC isozymes are expressed in glioma cells if the functions of these proteins are to be elucidated.

Xiao et al.⁸² have compared the levels of PKC isoforms in glioma lines versus "normal human glial cultures". They reported that while there was no increase in PKC α or γ between glioma cells and the "normal cells", PKC ϵ was increased 30 fold while PKC ζ was elevated 2 fold in glioma cells. It should be pointed out that there was no description of the characteristics of the "normal human glial cultures" and whether these were indeed characteristic of non-malignant human glia.⁸⁵

In summary, there is increasing evidence to implicate PKC in the abnormal growth of malignant glioma cells. Evidence provided by this laboratory is summarized in Table 1.

PKC AND OTHER ASPECTS OF GLIOMA BIOLOGY

Although we have focused on understanding the role of PKC in the growth of glioma and employed PKC inhibitors to decrease proliferation, this enzyme may serve a variety of other functions important in producing the malignant phenotype. One important emerging role for PKC is in the prevention of apoptosis, or programmed cell death.⁸⁶⁻⁸⁸ The mechanism by which transformed cells subvert the apoptotic program is not fully delineated but the expression of the bcl-2 protooncogene and mutation of the p53 tumor suppressor gene appear to be important. The expression of bcl-2 appears to be partially dependent on PKC, and it has recently been demonstrated that treating glioma cells in vitro with PKC inhibitors causes cytotoxicity with characteristics of apoptosis.⁸⁹ Given that different glioma cell lines have different levels of bcl-2 as well as different p53 status, it would be of interest to determine if there was a differential sensitivity of these cell lines to PKC inhibition. Also, among several glioma lines, there was differential expression of Fas/APO-1, which rendered these cells differentially susceptible to anti-fas/APO-1 antibody-mediated apoptosis.⁹⁰

Malignant gliomas are highly invasive tumors and their invasiveness appears to be related to the ability of proteolytic enzymes to degrade the surrounding extracellular matrix (ECM). It had been previously shown that staurosporine could inhibit invasion of bladder carcinoma⁴¹ and a recent study by

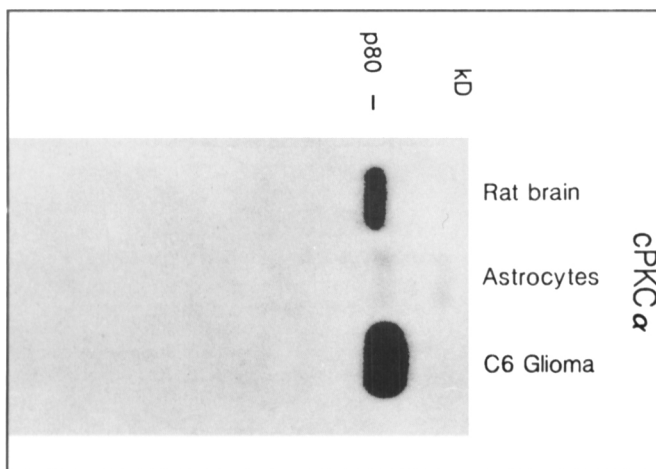


Figure 2: Western blot analysis to document the relative amounts of PKC α in the cytosol fraction of non-malignant neonatal rat astrocytes and C6 rat glioma cells, with rat brain as a positive control. 100 μ g total protein of each sample was loaded per lane. By phosphorimager analysis, the amount of PKC α in C6 is 59 fold higher than that of astrocytes.

Table 1: Summary of Results that Implicate PKC in the Growth of Malignant Gliomas.**In vitro:**

1. Human or rat glioma cells have high PKC enzyme activity when compared to their non-transformed glia counterparts.
2. Between several glioma lines, maximal PKC enzyme activity correlated well with their respective maximum rates of proliferation.
3. Within each glioma line at early, log or plateau phase of growth, the PKC activity at each phase correlates with the respective rate of proliferation.
4. High PKC activity and its correspondence with growth is selective to gliomas but not to other non-glial-derived cells or tumors.
5. Inhibitors of PKC block basal or mitogen-enhanced proliferation rates of glioma cells.
6. PKC α expression is increased at least 10 fold in C6 glioma cells when compared to rat astrocytes.
7. Antisense oligonucleotides to PKC α attenuate the proliferation rates of human or rat glioma cells.

In vivo:

1. Resected human glioblastoma specimens have high PKC enzyme activity.
2. Tamoxifen, when used at doses that can inhibit PKC, appears to have clinical utility in a significant proportion of patients with high grade gliomas.

Specific details of the above can be found in references 68, 69, 70, 72, 73 and 95.

this laboratory has demonstrated that the relatively selective PKC inhibitor, Calphostin C, causes a marked reduction in glioma invasiveness in an *in vitro* Boyden chamber invasion system.⁹¹ This finding may be highly clinically relevant as mortality in glioma is directly related to local recurrence.

The emergence of drug resistant tumor clones constitutes a serious challenge in cancer chemotherapy. Expression of multi-drug resistance has been reported in human gliomas and appears to be closely associated with the overexpression of P-glycoprotein, an energy-dependent pump that decreases intracellular drug accumulation.⁴² The biological function of this 170 kDa glycoprotein appears to be related to its state of phosphorylation of serine residues by PKC.⁹² Cells selected for multidrug resistance generally contain increased levels of PKC. Therefore inhibition of PKC could potentially act to modulate the sensitivity of cells to cytotoxic agents. Matsumoto et al.⁸⁴ have recently demonstrated that Calphostin C could act *in vitro* on glioma cell lines to reverse their resistance to vincristine.

Radiotherapy remains the cornerstone of adjuvant therapy for malignant gliomas. The dose of radiation is often limited by the undesirable side effect of radiation necrosis of surrounding brain. The development of radiosensitizers allows the delivery of lower doses of radiation with equivalent anti-tumor effect. It has recently been demonstrated that the *in vitro* treatment of C6 glioma cells with tamoxifen resulted in marked radiosensitization.⁹³

Thus, PKC appears to be critical in regulating many aspects of glioma biology (summarized in Table 2). The findings suggest that agents with anti-PKC activity can have utility in glioma therapy.

Table 2: Phenotype of glioma cells that may be regulated by high PKC.

Increased proliferation rate
 Increased invasive phenotype
 Decreased tendency for apoptosis
 Acquisition of multidrug resistance
 Decreased susceptibility to radiosensitisation

Whether each of these phenotypes is regulated by a specific PKC isoform remains to be determined.

PROTEIN KINASE C AS A TARGET FOR CHEMOTHERAPY

While targeting specific growth factor receptors that are known to be overexpressed on the cell membrane of glioma therapy, not all gliomas are known to harbor these abnormalities. Furthermore, the proportion of gliomas with defects of a specific growth factor receptor may be relatively small. For example, in the study by Lang et al.,¹⁹ gene amplification for EGFR was found in only 21% of glioblastoma multiforme, and in fewer still of lower grade gliomas. Given the evidence above implicating PKC in gliomas, targeting this intracellular pathway upon which different growth factors-initiated signals may converge could be more effective.

Tamoxifen, a triphenylethylene, is a known chemotherapeutic agent widely used in the adjuvant treatment of breast carcinoma. The agent is taken orally, is well tolerated, and readily crosses the blood brain barrier. Although primarily an estrogen receptor inhibitor, tamoxifen at much higher concentrations is also known to inhibit PKC.³⁷ Previous work has demonstrated that tamoxifen inhibits the proliferation of gliomas *in vitro*, independent of its action on the estrogen receptor;⁷¹ based on these findings, a human trial was started using conventional dose of tamoxifen (40 mg b.i.d.) as an adjuvant agent in the treatment of malignant glioma.⁹⁴ Work in our laboratory suggested that if the effect of tamoxifen on gliomas was through PKC inhibition, much higher plasma drug levels (μ M) would be necessary to inhibit the tumor than those attained by conventional therapy.⁹⁵ Therefore we, and others, began a protocol of high dose oral tamoxifen (150 mg/2m b.i.d.). The results from these trials are encouraging for a proportion of the patients,^{96,97} and presently, a Phase II trial is in progress.

The overexpression of PKC α in rat glioma versus non-transformed rat glia suggests that selectively targeting this isoform could provide a selective form of therapy. As yet, no pharmacological inhibitor specific for PKC α exists; however, using recombinant DNA technology, antisense oligonucleotides specific for PKC α may be designed to selectively inhibit translation of the PKC α protein. As described above, we have employed antisense oligonucleotides directed against the initiation site for PKC α translation and found that the proliferation rate of C6 glioma, as well as human glioma cell lines, was markedly reduced when compared to untreated controls, or to controls treated with random generated oligonucleotides of identical base composition.^{72,73} Ahmad et al.⁸³ have recently reported that an anti-sense construct to PKC α , when expressed in a glioma cell line, inhibited the growth and tumorigenicity of the cells.

In previous work, we have determined that only a proportion (30-40%) of glioma lines responded to tamoxifen treatment *in vitro* with a decrease in proliferation rate.⁹⁵ The reasons for the

lack of response to tamoxifen by the majority of glioma lines remain purely speculative at the present time; however, it is of interest to note that in clinical trials, a similar proportion of human patients do not respond to the tamoxifen therapy. Given this agreement between *in vivo* and *in vitro* data, it is of further interest that all glioma lines tested were inhibited in proliferation rate by more potent PKC inhibitors such as staurosporine and CGP 41251.^{70,95} Whether these more potent inhibitors will have improved clinical efficacy for glioma patients warrant testing.

CONCLUSION AND PERSPECTIVES

Although the concept of using PKC inhibition, whether it be by a pharmacological inhibitor or by antisense methods, as an adjuvant therapy is attractive, the importance of this enzyme in the normal functioning of the nervous system must be considered in designing any therapeutic strategy. Comparison of the effects of staurosporine on glioma cells and neonatal rat astrocytes indicates that staurosporine is marginally more effective in inhibiting proliferation of the malignant cells.⁹⁵ Whether this may provide a therapeutic window for effective chemotherapy without much associated cerebral toxicity remains unknown; adult human astrocytes have a very low rate of proliferation *in vitro* when compared with neonatal rat or fetal human astrocytes⁹⁸ and conceivably will be less susceptible to the anti-proliferative effects of a PKC inhibitor. As well, it must also be recognized that neurons also have high PKC activity, and that *in vivo*, neuronal function may be adversely affected by inhibitors of PKC. Phase I clinical trials with high dose tamoxifen have, however, demonstrated no significant deleterious effect on mental status; these results remain preliminary, and formal neuropathological testing has not been performed.^{96,97}

Finally, although tamoxifen is available for clinical use, it is a weak and non-selective inhibitor of PKC. As well, *in vitro* and *in vivo* findings suggest that a significant proportion of patients will not respond to this therapy.⁹⁵ More potent and selective inhibitors of PKC may have better efficacy in the treatment of patients with malignant gliomas and further assessment of this possibility is clearly warranted.

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