Protein kinase C links to cancer

The protein kinase C (PKC) enzymes are a group of serine/threonine kinases that are intimately involved in signal transduction pathways. This family of enzymes has been implicated in a variety of diseases including cancer, asthma and cardiovascular disorders, such as hypertension and atherosclerosis [Bradshaw, D. *et al. Agents Actions* (1993) 38, 135–147]. There are twelve known PKC isozymes, which may offer potential as therapeutic targets for a wide range of disease states [Gordge, P.C. *et al. Cell. Signal.* (1994) 6, 871–882].

Tumor activation

The involvement of PKC in signal transduction and thus its association with cell differentiation and growth has linked inappropriate PKC activation to tumor growth. The two-stage model of carcinogenesis, in which bound PKC is directly activated by 12-O-tetradecanoylphorbol-13-acetate (TPA) and similar phorbol esters known to be potent tumor promoters, clearly established a link between PKC activity and tumorogenicity [Niedel, J.E. *et al. Proc. Natl. Acad. Sci. U. S. A.* (1983) 80, 36–40]. In fact, it is now known that many clinically proven anticancer agents are either inhibitors or activators of PKC. For example, the PKC inhibitor staurosporine and various staurosporine analogs have proven efficacious in a variety of *in vitro* and *in vivo* tumor models [Harris, W. *et al. Drugs of the Future* (1993) 18, 727–735]. In contrast, the anticancer agent bryostatin is a potent activator of PKC *in vitro*, which uses the same binding site on PKC as the phorbol esters [Hennings, H. *et al. Carcinogenesis* (1987) 8, 1342–1346]. It acts by initiating tight membrane binding by PKC, which eventually leads to degradation of the enzyme itself. These examples correlate with the seemingly anomalous behavior of phorbol esters, which have been shown to either induce or inhibit cellular differentiation depending on the cell type.

The role of PKC in modulating MDR

More recently, PKC has been linked to the multiple drug resistance (MDR) exhibited by some cancers through its interaction with the plasma membrane protein P-glycoprotein (PGP). PGP is a product of the *mdr*1 gene, and has been linked to MDR through its ability to act as an ATP-dependent drug efflux pump. The mechanism of action of well-understood MDR-reversal drugs, such as verapamil and cyclosporin A, is simply to compete with the anticancer drug for PGP binding, and therefore retard efflux of the anticancer agent. The drawback of these reversal agents is acute toxicity, therefore novel MDR treatments without this side effect are highly desirable.

Although the action of PKC appears to be ambiguous, with reports of both increased and decreased drug sensitivities, this enzyme certainly offers a unique opportunity for the treatment of MDR. As phosphorylation of PGP by PKC activates PGP and leads to increased resistance in drug-resistant cell lines, attempts have been made to modulate PKC activity using phorbol esters. For example, Posada, J. A. and coworkers [*Cancer Commun.* (1989) 1, 285–292] showed that prolonged TPA treatment of wild-type cells and adriamycin-resistant S-180 cells led to increased resistance to adriamycin in both cell types. Paradoxically, TPA treatment of A253 carcinoma cells has been shown to sensitize them to cisplatin treatment. [Basu, A. *et al. J. Biol. Chem.* (1990) 265, 8451–8457].

A recent study details a unique approach to overcoming MDR in human-breast-cancer MCF7 and MCF7-MDR cell lines. Phorbol ester treatment of MCF7 cells causes PKC to phosphorylate PGP more efficiently, thus causing MDR by increasing the rate of the PGP efflux pump. Gupta, K.P. and coworkers [*J. Biol. Chem.* (1996) 271, 2102–2111] identified a novel pseudosubstrate of PKC-α (an *N*-myristoylated peptide), which blocks the PGP-phosphorylation site in MCF7 and MCF7-MDR cells, thereby inhibiting phosphorylation of PGP and increasing drug accumulation within the cells.

The variety of MDR responses elicited by different PKC-related stimuli are apparently due to a number of factors. Specific and relative levels of PKC isozymes present in the different cell lines, modes of drug action and differences in experimental protocols, such as short-term versus long-term phorbol ester exposure, all affect the PKC/MDR paradigm. These complexities are further exacerbated by the fact that many MDR cell lines display elevated levels of PKC when compared with the wild type. It is therefore apparent that individual tumor cell lines must be studied in some depth to establish the effect of PKC modulation on MDR in each case.

The future

While connections between PKC and cancer are indisputable, many questions remain unanswered. The intricate workings of signal transduction pathways coupled with variable tissue and cell distributions, not only of PKC as a family but of individual PKC isozymes, only serve to complicate an already challenging field. To date, the most well understood target of MDR and its links, in particular, to PKC-α have given new insight into areas of research that will continue to improve the understanding of cancer chemotherapies at the subcellular level.

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Inhibition of angiogenesis as a potential therapeutic strategy

Angiogenesis is defined as the growth of new capillary blood vessels, and plays a fundamental role in growth and development. In mature adults the ability to initiate an angiogenic response is present in all tissues, but is held under strict control. Normally, angiogenesis is only mobilized for wound repair, or in highly specific situations such as endometrial regulation. In these circumstances angiogenesis is very tightly regulated in both time and space. This overall situation of suppressed angiogenic potential relies on a very fine balance between numerous stimulatory and inhibitory factors including enzymes, growth factors and cytokines.

The role of angiogenesis in disease states

Inappropriate angiogenesis is now recognized as playing a crucial role in a number of disease states. These conditions include rheumatoid arthritis (blood vessels invade the normally avascular pannus),

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atherosclerosis (blood vessels grow into developing arterial plaques), diabetic retinopathy (capillaries invade the normally avascular eye), childhood hemangiomas (highly vascularized benign growths) and the growth and metastatic spread of solid tumours [Folkman, J. *Nat. Med.* (1995) 1, 27–31]. Following the pioneering work of Prof. Judah Folkman and coworkers (Harvard Medical School, Boston, MA, USA) we now know that solid tumours must constantly induce angiogenesis in order to secure the blood supply needed for continued growth. Indeed solid tumours cannot grow to above around 10^6 cells, or about the size of a pea, without inducing angiogenesis and thus establishing their own blood supply. In addition, the dissemination of cancer cells leading to secondary growths (metastasis) is closely linked to the ability of the primary tumour to induce angiogenesis, and thus its access to the vascular system, and these secondary tumours themselves can not grow successfully without inducing angiogenesis. As angiogenesis is not a continuous event in normal circumstances, molecules that can inhibit angiogenesis hold the promise of providing new noncytotoxic treatments for both primary tumours and secondary metastatic cancer, and of potentially providing new approaches to the treatment of other diseases that have an angiogenic component.

This thesis is reinforced by the work of Folkman and coworkers in the treatment of highly vascularized hemangiomas in young children with interferon-α (INFα). Long-term treatment with INFα inhibits the angiogenesis, which is a characteristic of these growths, and allows the natural regression of these lesions to occur much more quickly. The most likely mechanism for the action of INFα is based on the observation that it inhibits the production of the angiogenesis stimulatory factor, basic Fibroblast Growth Factor (bFGF). Treatment with INFα normalizes the abnormally high levels of bFGF in these children, and has saved the lives of many patients by slowing the angiogenesis-driven growth of the hemangioma and speeding natural regression.

Angiogenesis inhibitors

The endothelial cells that line blood vessels are among the slowest regenerating cells in the body, with normal cell-turnover times measured in years. During angiogenesis these endothelial cells are stimulated to proliferate much more rapidly, dividing in days and releasing a cocktail of growth factors and degradative enzymes. These stimulated endothelial cells then migrate towards the stimulus, eventually forming columns and then tubes of cells, and producing new capillary blood vessels. Based on the above rationale, medicinal chemists, biochemists and clinicians have searched for compounds that can inhibit these essential steps of angiogenesis, and several of these molecules are now in clinical trials.

TNP 470 (previously designated AGM 1470) is a semisynthetic compound derived from the natural product Fumagillin, which was discovered to be an angiogenesis inhibitor by Folkman's group. Takeda selected TNP 470 for clinical trials from a large number of synthetic analogues, based on a range of animal models indicating that TNP 470 is both less toxic and more potent than the parent Fumagillin. TNP 470 inhibits endothelial cell proliferation and migration, and shows pronounced antitumour effects in mice [Tanaka, T. *et al. Cancer Res.* (1995) 55, 836–839]. Phase I trials were initiated in late 1992, but have failed to show efficacy to date, and dose escalation is in progress. In addition, TNP 470 has now entered Phase I trials for chemotherapy-resistant relapsed childhood leukaemia, in which there is intense angiogenesis in the bone marrow, and for which there is currently no effective therapy.

Platelet factor 4 (PF4) is a natural component of platelet alpha granules, whose normal role is poorly defined. Recombinant PF4 has been shown to be angiostatic by workers at the Repligen Corp., and also to inhibit endothelial cell proliferation. Recombinant PF4 has now progressed to Phase II trials.

Thalidomide has been demonstrated recently to be orally active as an inhibitor of angiogenesis. In fact, the appalling birth defects caused by this compound may have been related to the inhibition of angiogenesis in the developing limb buds between the third and sixth weeks of pregnancy. As thalidomide is nontoxic (it is so well tolerated it does not have a measurable LD_{50}), and is orally active as an angiogenesis inhibitor, it has been entered directly into Phase II trials.

A number of other agents are currently in clinical trials as angiogenesis inhibitors, including interleukin-12 (Hoffman la Roche), Galardin (Glycomed) and BB 2576 (British Biotechnology). The effects of INFα are still being studied, and it continues to show remarkable effects in promoting the natural regression of hemangiomas in children.

Future targets

Basic research aimed at identifying new targets for angiogenesis inhibition also continues, with the integrins, which govern the interactions of endothelial cells with a variety of extracellular matrix components, currently attracting much attention. Specifically, $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins seem to play key roles in the survival of newly formed vascular cells, and antagonists of the binding of these integrins are inhibitors of angiogenesis. The race is on to develop small-molecule nonpeptide antagonists of these integrin interactions.

The results of the clinical trials currently under way, and undoubtedly of others yet to be launched, will determine if the hypothesis that the inhibition of angiogenesis is a valuable new strategy in cancer therapy is valid. The very nature of the therapeutic intervention may, however, make the demonstration of efficacy for angiogenesis inhibitors very difficult under traditional protocols, and combination therapy with other more classical agents, together with new approaches, may be necessary. The drugs emerging from these trials hold the hope of a new generation of nontoxic anticancer agents, and may offer new strategies for the treatment of other diseases whose common factor is the inappropriate growth of new blood vessels.

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Combinatorial chemistry

Antifungal analogues

Synthetic solid-phase chemistry now permits the preparation of a diverse range of drug molecules. A recent paper from Bristol-Myers Squibb describes the preparation of analogues of the antifungal compound miconazole (**1**) [Tortolani, D.R. and Biller, S.A. *Tetrahedron Lett.* (1996) 37, 5687–5690]. Hydroxymethylbenzoic acid was attached to Merrifield resin and reacted with *N*-iodosuccinimide and a

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