Tumour promoters

Protein kinase, phospholipid and control of growth

from I. Bernard Weinstein

THE mechanism of action of tumour promotors has until recently been largely mysterious. Unlike the initiating carcinogens, they cannot on their own induce cancer: but they can produce tumours when administered together with an ordinarily sub-carcinogenic dose of an initiating carcinogen. By contrast with the initiating carcinogens, which act directly on cellular DNA, many tumour promotors act primarily on receptors associated with cell membranes; but it has been unclear how. Recent studies suggesting that, in common with other growth-controlling agents, they may act through a specific protein kinase have therefore aroused considerable interest.

High-affinity saturable receptors for 12-*O*-tetradecanoyl phorbol 13-acetate (TPA) and structurally related plant diterpenes, as well as the recently discovered indole alkaloid and polyacetate classes of tumour promoter[1-4], have been identified in species ranging from nematodes to humans and in tissues as diverse as the skin, lymphocytes and brain.

It is clear that several of the structural and functional changes in cell membranes induced by the phorbol ester tumour promoters are mediated directly at the level of the cell membrane, since they occur within seconds or minutes of exposure and are not blocked by inhibitors of RNA and protein synthesis[1,2]. Presumably, the subsequent pleiotropic effects on growth and differentiation, some of which do require macromolecular synthesis, represent secondary effects triggered by the initial binding of the tumour promoters to the membrane-associated receptors[1,2].

Results reported by Castagna *et al.*[5], and later from other laboratories[6-8], now suggest that the phorbol ester tumour promoters act by binding to and activating a specific calcium- and lipid-dependent protein kinase, protein kinase C.

It is widely recognized that the post-translational phosphorylation of serine and threonine residues in specific cellular proteins has an important role in mediating the action of various hormones and growth factors. At least four classes of such protein kinase (all of which require Ca^{2+}) are known: cyclic AMP-dependent, cyclic GMP-dependent, calmodulin-dependent and phospholipid-dependent (protein kinase C)[9]. Certain oncogenes code for protein kinases that phosphorylate tyrosine residues; and tyrosine kinase activity is associated with the receptors for epidermal growth factor, insulin and platelet-derived growth factor[10].

The properties of protein kinase C are particularly intriguing, since this enzyme is ubiquitous in eukaryotes, it is strictly dependent on phospholipid (typically phosphatidylserine) and Ca^{2+}, and its activity is markedly enhanced by unsaturated diacylglycerol[5,9,11]. The latter findings suggest that the activity of this enzyme *in vivo* might be modulated by alterations in membrane structure and phospholipid metabolism, and thus it could play a key part in transmitting signals across membranes.

Castagna *et al.*[5] have found that low concentrations (about 10^{-8} M) of TPA can substitute for diacylglycerol in the activation *in vitro* of rat brain protein kinase C, apparently by enhancing the interaction of the enzyme with Ca^{2+} and phospholipid. Phorbol compounds that lack tumour-promoting activity are inactive and TPA does not activate the enzyme in the absence of phospholipid. Evidence has been obtained that TPA also enhances the activity of protein kinase C in intact platelets[5].

Niedel *et al.*[6] have confirmed the finding that TPA activates rat brain protein kinase C activity *in vitro*. Furthermore, using divalent ion chelation they solubilized a protein from rat brain membranes which co-purified with protein kinase C, and bound labelled phorbol ester, but only in the presence of added phospholipid. Other investigators[8,12] have detected a soluble protein in various murine tissues that binds tumour-promoting phorbol esters with high affinity and requires Ca^{2+} and phospholipid for maximum activity. The level of this protein in several murine tissues roughly parallels that of protein kinase C activity[8]. Curiously, the levels of phorbol ester-binding activity and protein kinase C activity are highest in brain.

It would appear, therefore, that protein kinase C may itself be a phorbol ester receptor, or at least a component of a receptor complex. Since, however, in the above studies neither the receptor nor the protein kinase was purified to homogeneity, further studies are required to establish that they are identical molecules.

TPA synergizes with, but does not substitute for, phospholipid in enhancing the activity of protein kinase C, and there is evidence that phorbol esters can bind with high affinity to synthetic lipid membranes and alter their physical properties, even in the absence of protein[13,14]. Despite the fact that their chemical structures are quite different, the tumour-promoting phorbol esters, the indole alkaloid teleocidin and the polyacetate compound aplysiatoxin have somewhat similar hydrophilic and hydrophobic domains[1,4,15]. Because of their amphipathic character they may, therefore, bind to specific domains in phospholipid matrices.

The resultant changes in lipid structure could, in the presence of Ca^{2+}, enhance the binding of phospholipid to the protein kinase C apoprotein. This could explain why, when intact cells are treated with TPA, soluble protein kinase C molecules seem to be translocated from the cytosol to membranes[7]. The hydrophilic domains of these tumour promoters could also interact specifically with the protein kinase C apoprotein to enhance the formation of a quaternary complex between phosopholipid, the protein kinase, the tumour promoter and Ca^{2+}.

Exposure of cells to phorbol esters, teleocidin or aplysiatoxin induces increased turnover of membrane phospholipids, presumably because of the activation of phospholipases including phospholipase C[1,4,15,16]. This could generate diacylglycerol, itself an activator of protein kinase C[5,11], thus further augmenting protein kinase C activity. In this sense the long-sought putative endogenous analogue of these tumour promoters[1] might be a diacylglycerol. Perhaps the well known effects of dietary lipid on carcinogenesis[1] also act by altering membrane properties that influence the activities of protein kinases. It will be of interest to determine whether tumour promoters might also act by modulating the activities of other enzyme systems that are lipid-dependent.

The hypothesis that certain tumour promoters act via lipid-dependent protein kinases provides a satisfying unity to current research on growth factors, chemical carcinogenesis and tumour virology. In all cases, changes in protein phosphorylation may play a key part, although the precise kinases and target proteins that become phosphorylated may differ considerably. □

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