Nephrol Dial Transplant (1994) 9: 1061–1062

Nephrology
Dialysis
Transplantation

Editorial Comment

Regulatory functions of protein kinase C isoenzymes in glomerular mesangial cells

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Protein kinase C (PKC) is a family of isoenzymes that are activated by hormone-stimulated phosphoinositide and phosphatidylcholine hydrolysis and participate in the signalling process by phosphorylating certain target proteins. The sustained activation of PKC seems to be essential for maintaining cellular responses such as cell growth and differentiation. PKC also plays a pivotal role in the pathogenesis of inflammation, and activation of PKC by phorbol esters causes all major signs of inflammation including redness, heat, swelling and pain. The contribution of individual PKC isoenzymes to these processes is still ill defined.

The mesangium is a highly specialized pericapillary tissue that is involved in most pathological processes of the renal glomerulus. The cross-talk between intrinsic glomerular cells and invading macrophages and T-cells is of central importance in the pathogenesis of glomerular injury. The progress of *in-vitro* cell culture techniques, particularly with regard to the glomerular mesangial cells, has greatly promoted our understanding of the relevant pathomechanisms. Three prominent proinflammatory features of intrinsic mesangial cells evolve as a result of the cross-communication with invading immune cells: (a) increased eicosanoid and other mediator production, (b) increased matrix production by mesangial cells, and (c) increased mesangial cell proliferation.

We have used rat glomerular mesangial cells to study two major control functions exerted by PKC: the regulation of hormone-stimulated prostaglandin synthesis and the negative feedback regulation of the inositol lipid signalling cascade [1]. Using specific antibodies for PKC isoenzymes, we compared the time courses of down-regulation of isoenzymes and functional cell responses. We observed that mesangial cells express four PKC isoenzymes, PKC-α, -δ, -ε and ζ. Neither PKC-β, -γ or -η were detected [2–5]. Our experimental data led us to suggest that PKC-α mediates feedback inhibition of phosphoinositide hydrolysis, whereas PKC-ε is a candidate for stimulating PLA_2.

One important next step is the identification of the physiological substrates for PKC that mediate the observed cellular functions. The cellular target of PKC action responsible for the inhibition of angiotensin-II-stimulated IP_3 generation is not precisely known. However, there is evidence suggesting that the coupling between the angiotensin II receptor and the transducing G protein might be impaired [6].

The hormone-induced release of arachidonic acid from membrane lipids is the rate-limiting step in the synthesis of eicosanoids. The dominant factor regulating arachidonic acid liberation in most cells, including mesangial cells, is phospholipase A_2 (PLA_2). Two types of PLA_2 have been described in mesangial cells. One type is a low-molecular-mass (14 kDa) PLA_2 belonging to group II of the secretory PLA_2 ($sPLA_2$) that is induced and secreted from the cells upon stimulation with proinflammatory cytokines, such as interleukin 1 or tumour necrosis factor α [7]. Mesangial cells contain an additional PLA_2 that shows an apparent molecular mass of 110 kDa upon SDS/PAGE. Most interesting is the fact that the activity of the latter enzyme can be increased by preincubation of the cells with vasopressin or phorbol ester, indicating that this is a hormone-sensitive cellular PLA_2 ($cPLA_2$) and that PKC can increase its activity.

The presence of potential phosphorylation sites for PKC in the sequence of $cPLA_2$, but not $sPLA_2$, indicate that the former enzyme might be phosphorylated by PKC-ε in mesangial cells. A selective inhibitor for PKC-ε is therefore expected to act as a functional $cPLA_2$ inhibitor and to suppress formation of proinflammatory eicosanoids and PAF. The endogenous activator of PKC, DAG, has an extremely short half-life due to rapid metabolism. We have recorded a biphasic sustained rise in DAG formation in mesangial cells during stimulation with angiotensin II, platelet-derived growth factor or extracellular nucleotides. The source of DAG produced during the later phases of hormone stimulation are derived from a substantial breakdown of phosphatidylcholine by a phospholipase D [8,9] and may contribute to a sustained activation of PKC. Phorbol esters, which can activate PKC, are able to stimulate PLD-mediated phophatidylcholine degradation in mesangial cells. Furthermore, down-regulation of PKC eliminates angiotensin-II- and ATP-stimulated phophatidylcholine breakdown, thus sug-

gesting that PKC-ε is a candidate for triggering phospholipase D activation in mesangial cells [8,9]. PKC-ε activation of phospholipase D may provide a possible positive feedback loop on PKC regulation and may trigger long-term cell responses, e.g. mesangial cell proliferation.

The mesangial cell in culture provides an excellently characterized cell culture system with well-known signalling pathways. Functional identification of PKC isoenzymes involved in specific cell responses is one of the most promising steps towards understanding of the molecular mechanisms of cell regulation. So far, rational drug design targeted towards PKC has mainly focused on the association between PKC activation and growth modulation, and PKC was considered as a target for new anticancer drugs. However, PKC is also a crucial regulator of proinflammatory cellular processes and thus may become a target for anti-inflammatory therapy. This may lead to the development and design of potent, isoenzyme-specific inhibitors of PKC for the treatment of glomerulonephritis and other inflammatory and autoimmune diseases.

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