A Ca\textsuperscript{2+}-Independent Protein Kinase C, nPKC\textsubscript{\eta}: Its Structure, Distribution and Possible Function

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Department of Cancer Cell Research, Institute of Medical Science, University of Tokyo, Tokyo 108, and *Department of Molecular Biology, Yokohama City University, School of Medicine, Yokohama 236

HASHIMOTO, Y., OSADA, S., OHNO, S. and KUROKI, T. A Ca\textsuperscript{2+}-Independent Protein Kinase C, nPKC\textsubscript{\eta}: Its Structure, Distribution and Possible Function. Tohoku J. Exp. Med., 1992, 168 (2), 275-278 — Protein kinase C consists of a protein family which can be classified into two major groups: Ca\textsuperscript{2+}-dependent conventional protein kinase C and Ca\textsuperscript{2+}-independent novel protein kinase C (nPKC). Among eight known members of protein kinase C family, we found that nPKC\textsubscript{\eta} (eta) isolated from cDNA library of mouse skin, is most abundant in epithelial tissues including skin and epithelia of digestive and respiratory tracts. These data suggest potential role of this isoform in growth, differentiation and carcinogenesis of epithelial tissues. — protein kinase C; tumor promotion; epithelial cells

Activation of protein kinase C

Protein kinase C (PKC) is a serine/threonine protein kinase which is considered to function as a critical component in the signal transduction pathways that are elicited by a variety of extracellular signals including hormones, growth factors, neurotransmitters and tumor promoters. PKC was first isolated in 1977 by Y. Nishizuka and his colleagues at Kobe University as a proenzyme of cAMP/cGMP-independent protein kinase (Takai et al. 1977). First, PKC was found to be activated in a Ca\textsuperscript{2+}-dependent manner in the presence of lipidsoluble factors in the membrane. Phosphatidylserine (PS) was found the most effective lipid among naturally occurring phospholipids. Subsequently, diacylglycerol (DG), a neutral lipid, was found to activate PKC in a PS- and Ca\textsuperscript{2+}-dependent manner at physiological Ca\textsuperscript{2+} concentrations. This discovery linked PKC to major signal transduction pathways involving phosphatidylinositol (PI) turnover, as DG is a metabolite of PI turnover. Castagna et al. (1982) found that low concentrations of phorbol esters could replace a requirement for DG to stimulate directly PKC.
suggesting that PKC itself is a receptor for phorbol esters. This discovery explained the pleiotropic effects of phorbol esters on cell growth, differentiation and also tumor promotion.

**Family members of PKC**

When the activity of PKC was measured biochemically, PKC was thought as a single enzyme. However, molecular cloning studies, reported since 1986, revealed that PKC is not a single protein but consists of a protein family, which can be classified into two major groups; conventional Ca\(^{2+}\)-dependent PKC (cPKC or simply PKC) and Ca\(^{2+}\)-independent novel PKC (nPKC). As shown in Fig. 1, conventional PKC contains four isoforms, PKC\(\alpha\), PKC\(\beta I\), PKC\(\beta II\) and PKC\(\gamma\) which are characterized by the requirement of Ca\(^{2+}\), phospholipids and DG (or TPA) for activation and structurally by the three conserved regions, i.e., regulatory C1 and C2 regions and catalytic C3 region. nPKC members are independent of Ca\(^{2+}\) for enzymatic activation and lack the C2 putative Ca\(^{2+}\)-binding domain in their structure. Although three isoforms (nPKC\(\delta\), nPKC\(\epsilon\), nPKC\(\zeta\)) are previously known for nPKC isoforms, we have cloned a new PKC member, termed nPKC\(\eta\) (eta) (Osada et al. 1990). Isolation of a new isoform is due to the use of a skin cDNA library, as most of previous cloning studies used brain cDNA libraries because of abundance of PKC in brain.

![Fig. 1. Schematic structure of members of the PKC family. Ca\(^{2+}\)-dependent conventional PKC consists of the isoforms of \(\alpha\), \(\beta I\), \(\beta II\), and \(\gamma\) and contains the regulatory C1 and C2 regions and the catalytic C3 region. Four isoforms, i.e., \(\delta\), \(\epsilon\), \(\zeta\), and \(\eta\) are known for Ca\(^{2+}\)-independent novel PKC which lack the putative Ca\(^{2+}\)-binding C2 region.](image-url)
Structure and distribution of nPKC\(\eta\)

nPKC\(\eta\) contains a characteristic cysteine-rich repeat sequence in the C1 region and a protein kinase domain sequence in the C3 region, both of which are conserved among PKC family members. Like other nPKC isoforms, however, it lacks the putative Ca\(^{2+}\)-binding C2 region. Homology of nPKC\(\eta\) with the other members of the PKC family is summarized in Table 1. Of the PKC isoforms,
nPKC\(\eta\) shows the highest sequence similarity to nPKC\(\epsilon\) (Osada et al. 1990).

We demonstrated that nPKC\(\eta\), like other members of the PKC family, has phorbol ester binding activity and autophosphorylates when stimulated by phorbol esters (Osada et al. 1990). For this study, we used COS cells expressing transiently the nPKC\(\eta\) cDNA, from which the product of the gene was immunoprecipitated by antiserum against a synthetic COOH-terminal peptide. These data suggest that nPKC\(\eta\) is able to act as a receptor for phorbol esters and as a protein kinase in transducing signals.

Northern blotting of total RNAs from various mouse tissues and embryos (Fig. 2) suggests the possibility that nPKC\(\eta\) is an epithelial-tissue specific PKC isoform. To test this hypothesis, we examined expression of each PKC isoform in skin and epithelia of digestive and respiratory tracts at the mRNA levels by Northern blotting and in situ hybridization and at the protein level by immunoblotting and immunohistochemistry (unpublished). We found that nPKC\(\eta\) is predominantly expressed, to much higher extents than in brain, in the epithelia of skin, esophagus, forestomach, glandular stomach, intestine, colon, trachea and bronchus. PKC\(\alpha\) and nPKC\(\delta\) are commonly present in these tissues at varying extents but no signals were detected for PKC\(\gamma\).

Our results indicate that Ca\(^{2+}\)-independent nPKC\(\eta\) is a major isoform of PKC which is expressed in epithelial tissues, suggesting its significant role in growth, differentiation and carcinogenesis in epithelial tissues.

References

