Protein kinase C in melanoma

Masahiro Oka1,∗ and Ushio Kikkawa2,†
1Division of Dermatology, Department of Clinical Molecular Medicine, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan; 2Biosignal Research Center, Kobe University, Kobe 657-8501, Japan

Key words: protein kinase C (PKC), phorbol ester, TPA, growth, metastasis

Summary

Protein kinase C (PKC) is activated by diacylglycerol generated by receptor-mediated hydrolysis of membrane phospholipids to mediate signals for cell growth and plays as a target of tumor-promoting phorbol esters in malignant transformation. PKC is a family of enzymes and their expression profiles have been examined in the normal melanocytes and melanoma cells, and studies have been carried out on the functions of PKC isoforms in proliferation, transformation, and metastasis of melanoma cells. Here, we summarize current knowledge of the expression and possible roles of the PKC family in melanoma in comparison with those of normal melanocytes.

1. Introduction

1.1. Background

Cancer is a disorder caused by alterations of genome such as mutations of dominant oncogenes and recessive anti-oncogenes with gain and loss of function, respectively, and tumorigenesis is a process composed of multiple steps in which the changes of these genes occur [1]. Protein kinase C (PKC) is established to play crucial roles for the regulation of cell growth [2,3], and has been studied extensively in melanoma cells along with well-established oncogenes and anti-oncogenes [4].

1.2. Discovery of PKC

In 1977, a novel protein-serine/threonine kinase was identified in bovine brain, from which a catalytically active fragment is generated by the cleavage with Ca2+-dependent protease [5]. During the analysis of the Ca2+-dependent protease, crude membrane phospholipids were revealed to replace the role of the protease to activate this enzyme. Namely, its protein kinase activity was enhanced by phospholipids without proteolysis in the presence of Ca2+ [6]. This protein kinase was thus named as Ca2+-activated, phospholipid-dependent protein kinase, for short protein kinase C (PKC), because Ca2+ was assumed to be an intracellular second messenger for this enzyme. In 1980, further analysis indicated that diacylglycerol, a neutral lipid produced in receptor-coupled hydrolysis of inositol phospholipids, increases the affinity of this enzyme for both Ca2+ and phospholipids [7]. In other words, it was estimated that PKC in cytosol is activated upon cell stimulation by the association with membrane phospholipids through Ca2+, when the intracellular Ca2+ concentration is elevated and diacylglycerol is generated in the plasma membrane. Concomitantly, PKC was shown to be expressed not only in brain but also widely among mammalian cells and tissues. Based on these results, PKC was proposed to be a target of diacylglycerol, which was recognized as a novel intracellular messenger produced in the signaling pathway of various biologically active substances [2]. Importantly, tumor-promoting phorbol esters such as 12-O-tetradecanoylphorbol-13-acetate (TPA) were found to activate PKC directly in a manner analogous to diacylglycerol in 1982 [8].
1.3. Heterogeneity of PKC

In 1986, molecular cloning and biochemical analysis indicated that PKC comprises a family of related proteins [9,10]. Today, ten PKC isoforms are identified as shown in Figure 1, that are conserved among different mammalian species. The PKC isoforms have the regulatory and catalytic regions in their amino- and carboxyl-terminal halves, respectively, and are classified into three groups according to the structure of the regulatory domain: cPKC (classical or conventional PKC) of α, βI, βII, and γ isoforms, nPKC (new or novel PKC) of δ, ε, η, and θ isoforms, and aPKC (atypical PKC) of ζ and λ/ι isoforms. The cPKC group has the C1 and C2 domains in the regulatory region. The C1 domain has a tandem repeat of cysteine-rich sequences that associate with diacylglycerol and phorbol ester, and the C2 domain has the Ca\(^{2+}\)-dependent phospholipid-binding sequence. Two isoforms, βI and βII, are the splicing variants derived from the same gene. The enzyme analyzed in the initial studies belongs to this group. The proteins encoded by the clones of the cPKC group reflect the enzyme, which had been studied in the primary studies, and later the PKC family was extended to find the following two groups. The nPKC group has the C1 and C2-like domains in the regulatory region. The C1 domain has a tandem repeat of cysteine-rich sequences that associate with diacylglycerol and phorbol ester, and the C2 domain has the Ca\(^{2+}\)-dependent phospholipid-binding sequence. Two isoforms, βI and βII, are the splicing variants derived from the same gene. The enzyme analyzed in the initial studies belongs to this group. The proteins encoded by the clones of the nPKC group reflect the enzyme, which had been studied in the primary studies, and later the PKC family was extended to find the following two groups. The aPKC group has a single cysteine-rich sequence in the C1 domain that does not associate with diacylglycerol or phorbol ester. The activation mechanism of the aPKC group isoforms is thus distinct from that of the cPKC and nPKC enzymes. Some PKC isoforms are activated not only by diacylglycerol but also by fatty acids and its metabolites at least in vitro, although the effects of these lipid compounds vary among the isoforms. In addition, the PKC isoforms appear to show different distribution among cells and tissues. The α, δ, and ζ isoforms are found in almost all cells examined. In contrast, the γ isoform is found only in the neuronal cells in the central nervous tissue. The expression of other isoforms is restricted depending on the cell types. The β, ε, and λ/ι isoforms are found in various tissues, and η and θ isoforms are predominant in epithelial and immune cells, respectively. The diverse enzymatic properties and tissue-specific distribution of the PKC family members suggest that each isoform plays a distinct role in the cellular signaling, and therefore, extensive studies have been carried out to clarify the roles of each PKC isoform in processing and modulation of physiological and pathological responses to external signals.

1.4. Melanoma and PKC

For the biological study of melanoma, it was essential to establish the method of the long-term cultivation of the normal melanocytes, the progenitor of melanoma cells. The attempt had been unsuccessful, but it became possible to grow and maintain the human melanocytes using the culture medium containing TPA in 1982 [11]. It was timely that, in the same year, PKC was revealed to mediate the action of phorbol ester [8]. Paradoxically, phorbol ester inhibits growth of most melanoma cells [12–14]. Since these discoveries, much attention has been directed to the roles of PKC, and later to those of the PKC isoforms, in the growth regulation of melanoma cells. For example, the roles of the PKC isoforms in melanoma have been reviewed for α [15,16], β [17], and δ [18,19] isoforms.

2. Expression of PKC isoforms in melanocytes and melanoma cells

2.1. Expression in melanocytes

The expression of mRNA and protein of the PKC isoforms has been studied in cultured normal human melanocytes as summarized in Table 1 [13,20–29]. Although the results differ slightly among the reports, the α, δ, and ζ isoforms, that are expressed ubiquitously among the cell types, are detected mostly in the cultured melanocytes. In addition, the β, ε, and λ/ι