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Novel PKC Localisation in Basic Condition and Subcellular Translocation after PMA Activation

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Abstract: We applied a subcellular fractionation of three cell lines (HEL, K562 and Jurkat). This technique based on successive centrifugation allows as to obtain three fractions; Cytosolic Fraction (FC), Membranous Fraction (FM) and Cytoskeletic Fraction (FX). The purpose of this study was to characterize the isoforms translocation and properties of PKC present in each cell line. Immunoblotting techniques were used to identify which individual PKCn isoforms were present in these cells in basic condition and its translocation from fraction to other after activation by the PMA (phorbol 12-myristate 13-acetate) as DAG analogue. UV irradiations indicate PKC localisation in cell apoptosis. The presence of each isoform in certain cells and not in the others indicates its specialized role and a limited implication in tissues where it is expressed. In leukaemic cell lines, the isoforms which seem us interested are PKCθ. PKCθ is expressed only on T cells and the muscular cells. This isoform from novel PKC can be regarded as target in therapy of human leukaemia.

Key words: Protein kinase C, PKCθ, PMA, leukaemia, subcellular fractionation, immunoblotting

INTRODUCTION

The Protein Kinase C (PKC) was discovered more than 20 years ago by Nishizuka (1986) as a histone protein kinase in rat brain tissues that could be activated by limited proteolysis. Members of the PKC family of signal transduction molecules have been widely implicated in regulation of cell growth/cell cycle progression and differentiation (Matsumura *et al*., 2003). Increasing evidence from studies using *in vitro* and *in vivo* systems points to PKC as a key regulator of critical cell cycle transitions, including cell cycle entry and exit and the G1 and G2 checkpoints. PKC-mediated control of these transitions can be negative or positive, depending on the timing of PKC activation during the cell cycle and on the specific PKC isozymes involved (Mark *et al*., 2000). According to their structural organization and their sensitivity to Ca^{2+} diacyl glycerols (DAGs), these isoforms have been classified into three groups: the classical cPKCs (α, βI, βII and β) require Ca^{2+} and DAG for full activation, while the new nPKCs (δ, ε, η, θ and μ) is activated by diacyl glycerols and atypical aPKCs (ξ, λ) are Ca^{2+} and DAG independent (Nishizaka, 1992) (Fig. 1). Members of the PKC family have been shown to regulate cell death by apoptosis. Several PKC isozymes, particularly novel PKC group are substrates for caspases (Alakananda and Usha, 2007; Siva Prasad *et al*., 2007).

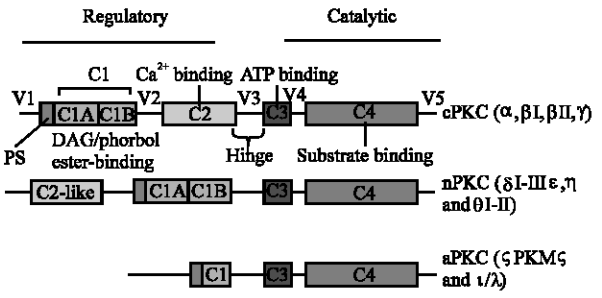

Fig. 1: Classification of Protein Kinase C family

In this study, we are interested in novel PKC (PKCε, PKCα, PKCη and PKCθ).The DAG drives the translocation of the inactive cytoplasmic nPKC to the membrane (Kraft and Anderson, 1983) and/or cytoskeletic elements (Mochly-Rosen *et al*., 1990). However it's noticed that PKC isoforms have a selective expression in body tissues (Wilda *et al*., 2001). The central aim is to characterize nPKC isoform expression in three leukaemic cell lines (HEL, K562 and Jurkat) in order to identify the suitable isoform as therapy target for leukaemic

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proliferation. The purpose of this study was to characterize the translocation and properties of PKC isoforms present in each cell line.

MATERIALS AND METHODS

Cell lines: We applied a subcellular fractionation of three cell lines (HEL, K562 and Jurkat). HEL (Human Erythro Leukemia), K-562 and Jurkat (JA16 and JE6.1) were maintained in exponential growth at 1 $U10^6$/mL in RPMI 1600 completed medium. Cells were then washed twice with cold PBS for subcellular fractionation.

Subcellular fractionation: Cells were resuspended in ice-cold hypotonic buffer as described by Magdalena *et al.* (2001).

To obtain stimulated fraction we stimulated cells either with phorbol myristate acetate (PMA, 50 ng mL^{-1}) and to study apoptosis involvement we irradiate cell lines fraction with UV.

Each fraction were centrifuged at 200 x g for 10 min to remove nuclei and cell debris and the supernatant was collected and centrifuged at 13,000 x g for 60 min at 4°C. The supernatant (cytosol) was collected and the pellet was resuspended in lysis buffer (20 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1% NP-40 and 10 μg mL^{-1} each aprotinin and leupeptin), vortexed for 5 min at 4°C and centrifuged again at 13,000 x g for 60 min at 4°C. The supernatant representing the particulate (membrane) fraction was saved and the detergent-insoluble fraction (cytoskeleton) was resuspended in 1% SDS in water. Each fraction was then diluted to a final concentration of 1% Laemmli buffer and separated by SDS-PAGE.

Immunoblot analysis: Cell line extracts were fractionated by SDS-PAGE, transferred to polyvinylidene difluoride membrane and probed with the appropriate antibody at 1:1000 dilution, antibody. Primary antibody was detected by horseradish peroxidase-conjugated secondary antibody which was used at 1:5000 and the enhanced chemiluminescence system (Amersham). Immunoblot were used to identify which individual PKCn isoforms were present in these cells in basic condition and it is translocation from fraction to other after activation by the PMA as DAG analogue. UV irradiations indicate PKC localisation in cell apoptosis.

RESULTS AND DISCUSSION

Generally, the activation of the PKC requires the translocation of the enzyme from the cytoplasm towards the plasmic membranous and in certain case towards the cytoskeleton. It is noticed that the level of expression of each isoforms is not the same for different cell lines. That is explained by the selective expression of each isoforms and with the role quite specialised to play thereafter in each tissue. Table 1 resume the presence (+) and absence (-) of different isoforms in each cells.

Table 1: PKC expression in HEL, Jurkat and K562 cell lines

	HEL	JURKAT	K562
PKCα	+	+	+
PKCε	+	-	-
PKCθ	+	+	+
PKCη	-	+	-

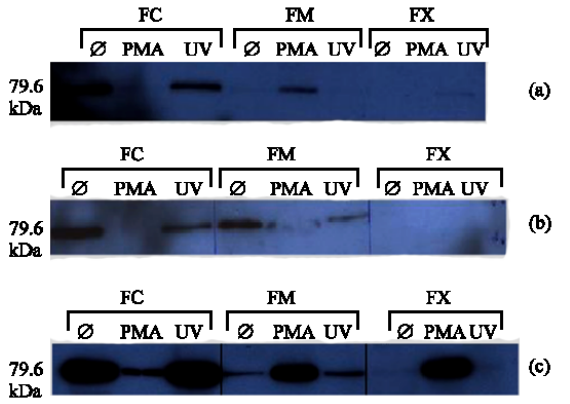

Fig. 2: Immunoblot analysis of PKCα: PKCα expression in basic condition (Ø), after PMA activation and UV irradiation in cytosolic (FC), membranous (FM) and Cytoskeletic Fraction (FX). (a) PKCα expression in HEL cells, (b): PKCα expression in Jurkat cells and (c): PKCα expression in k562 cells

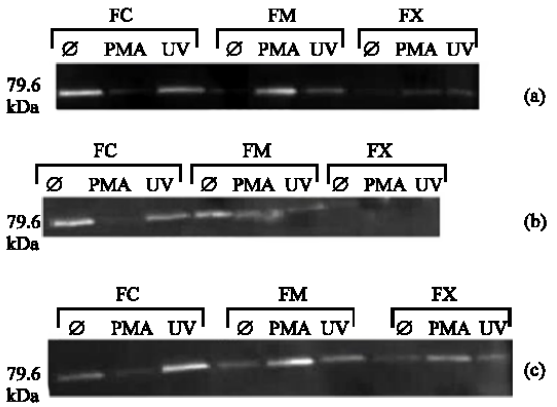

Fig. 3: Immunoblot analysis of PKCθ: PKCα expression in basic condition (Ø), after PMA activation and UV irradiation in cytosolic (FC), membranous (FM) and cytoskeletic fraction (FX). (a) PKCθ expression in HEL cells, (b): PKCθ expression in Jurkat cells, (c): PKCθ expression in k562 cells

From immunoblotting assay we can conclude that no translocation is associated to PKCα in jurkat cells (Fig. 2b). However it's translocated after PMA activation

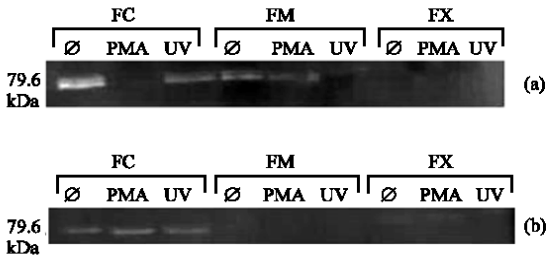

Fig. 4: Immunoblot analysis of PKC (ε, η): PKC (ε, η) expression in basic condition (Ø), after PMA activation and UV irradiation in cytosolic (FC), membranous (FM) and cytoskeletic fraction (FX). (a) PKCε expression in HEL cells, (b): PKCη expression in Jurkat cells

to the membranous fraction in HEL cells (Fig. 2a) and to the membranous and cytoskeletic fraction in K562 cell line (Fig. 2c).

PKCθ is contained in the cytosolic fraction. The activation with PMA induce its translocation to the cytoplasmic membranous (Fig. 3a-c) and to the cytoskeletic fraction in k562 cell line (Fig. 3c).

On the other hand with PKCε and PKCη the level of expression it's too little, a light translocation to the membranous is associated to PKCε in HEL cells (Fig. 4a). PKCη has a ubiquitous expression in jurkat cells (Fig. 4b).

CONCLUSION

The presence of each isoform in certain cells and not in the others indicates its specialized role and a limited implication in tissues where it is expressed. In present study we are interested in leukaemic cell lines, the isoforms which seem us interested and which are expressed in leukaemic cells are PKCθ and PKCα. The later PKCα has an universal expression (Iwamoto *et al*., 1992). Whereas PKCθ is expressed only on T cells and skeletal muscle cells (Pfeifhofer *et al*., 2003).

PKCθ can be regarded as target in therapy of human leukaemia (Hayashi and Altman, 2007). This objective is based on recent observations which showed that PKCθ intervenes in the signal transduction of survival in T cells, thus protecting them from the apoptosis induced by agents damaging DNA or by irradiations with UV the use of inhibitor of PKCθ could thus abolish the mechanisms of survivals and involve the apoptosis in order to eliminate the leukaemic T cells.

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