PICOT: A Trx- and Grx-Like Protein in Search of a Function

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Abstract: The PKC-interacting cousin of thioredoxin (PICOT) protein was discovered based on its ability to bind PKCθ in human T lymphocytes. Overexpression of PICOT was found to impose negative regulatory effects on PKCθ-dependent functions. This included the inhibition of PKCθ-dependent activation of c-Jun N-terminal kinase (JNK) and the activator protein 1 (AP-1) and nuclear factor kappa B (NF-κB) transcription factors. PICOT is a modular protein consisting of a single thioredoxin (Trx)-like homology domain (HD) and two highly homologous PICOT-HDs. The overall structure of each of the three domains resembles the canonical thioredoxin fold, which is common in enzymes that catalyze disulfide bond formation. Nevertheless, the three PICOT domains lack essential catalytic cysteine residues and their mode of activity is therefore unclear. PICOT is involved in the regulation of heart muscle function. Its overexpression in the heart of transgenic mice increased the ventricular function and cardiomyocyte contractility, and inhibited the overall cardiac hypertrophy induced by pressure overload. The effects of PICOT on the cardiac tissue are likely to be mediated *via* the muscle LIM protein (MLP), which was shown to interact with PICOT in cardiomyocytes, and colocalize with PICOT at the Z-disc of the sarcomer. PICOT interaction with MLP interfered with binding of the latter protein to the Ca^{2+}-dependent Ser/Thr phosphatase, calcineurin, causing the displacement of calcineurin from the Z-disc. As a result, PICOT inhibited the calcineurin-mediated dephosphorylation and nuclear translocation of nuclear factor of activated T cells (NF-AT), and the transcriptional activation of NF-AT regulated genes. Whether the effects of PICOT are dependent on PKC, and whether it can mediate catalytic activity and/or operate as an adaptor protein are only few of the open questions related to the biological mechanism of action of PICOT.

INTRODUCTION

Studies aimed at the identification of protein kinase C (PKC) theta (PKCθ) regulatory molecules, have led Witte *et al*. to the discovery of a new gene product, termed PICOT (PKC-interacting cousin of thioredoxin), which was found to possess the ability to associate with PKCθ in human T lymphocytes [1]. PICOT was first isolated using a yeast-two hybrid screen of human Jurkat T cell cDNA library with bait that consisted of a catalytically inactive full-length PKCθ cDNA.

The human PICOT predominates in the cell's cytoplasm; it has a molecular mass of ~37kDa and includes 335 aa. Its N-terminal domain share sequence homology with members of the thioredoxin family, but is missing a conserved motif (Cys-Gly-Pro-Cys) essential for the thioredoxin catalytic activity. It mediates however binding to PKCθ. The C-terminal includes a tandem repeat of an evolutionary conserved domain, termed PICOT-HD, which is shared by proteins from diverse groups of organisms (Fig. **1**) [2].

Transient overexpression of constitutively active PKCθ in Jurkat T cells resulted in upregulation of the c-Jun N-terminal kinase (JNK) activity, an effect that was partially inhibited by overexpressed PICOT. In addition, PICOT inhibited JNK activation induced by cotransfected constitutively active PKCθ and calcineurin, which are known to cooperate in the activation of JNK in antigen stimulated T cells [3]. Further studies demonstrated that PICOT inhibits PKCθ-dependent transcriptional activation mediated by the AP-1 and NF-κB transcription factors [1].

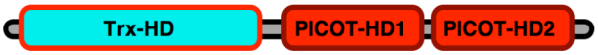

Fig. (1). A schematic structure of the human PICOT protein. The protein is 335 aa long and includes three conserved domains: an N-terminal thioredoxin (Trx)-like homology domain (HD)(aa12-143), and two 84 aa long PICOT-HDs (aa 145-228 and 247-330) that exhibit 64% identity in sequence. PICOT is also termed 'thioredoxin-like 2' (TXNL2) by the HUGO gene nomenclature, and 'glutaredoxin 3' (Glrx3) by the Mouse Genome Informatics Database.

T cell staining with PICOT specific antibodies (Abs) demonstrated that the protein is expressed predominantly in the cytoplasm. In addition, our own studies revealed that overexpression of PICOT in different cell lines resulted in predominant localization of the transfected gene product in the cytoplasm, with relatively low levels (that differ from one cell line to another) found in the nucleus (see Fig. **2**). Recent studies by Park and colleagues [4, 5] demonstrated

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that PICOT has a significant regulatory role in heart muscle function where it can attenuate stress-induced cardiac hypertrophy. The present review highlights some of the most recent findings on PICOT and discusses the potential biological function of this protein.

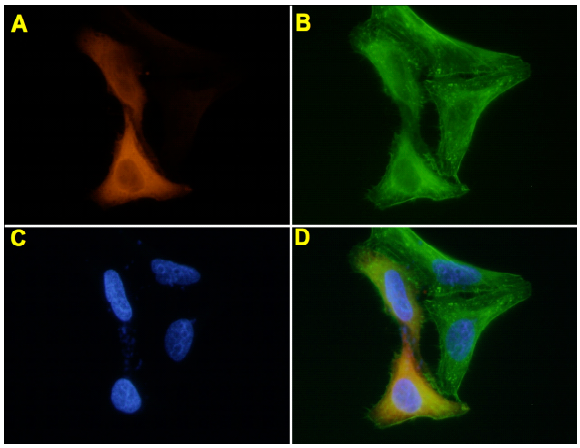

Fig. (2). Localization of overexpressed PICOT in HepG2 cells by immunofluorescence staining. HepG2 cells were transfected with pEF-PICOT, cultured for 3 days, fixed, and stained with rabbit anti-PICOT Abs and a PE-conjugated anti-rabbit IgG (**A**), phalloidin that specifically binds filamentous actin (**B**), and DAPI, which associates with the minor groove of the double strand DNA (**C**). A merged image is shown in **D**. PICOT localize predominantly in the cell's cytoplasm, with low levels at the nucleus.

STRUCTURE OF PICOT AND RELATIONSHIP TO THIOREDOXIN AND GLUTAREDOXIN

The biological role of PICOT has not been identified yet, nor is the exact physiological function of its isolated Trx- or PICOT-HD. Many different proteins from a wide range of species were found to include one or more PICOT-HD as part of the molecule. In a large number of such proteins, the PICOT-HD was found at the protein's C-terminus, in close proximity to N-terminal Trx- or Grx-like domains [2]. The close physical proximity between PICOT-HD and Trx- or Grx-HD in multiple proteins suggests functional relationships between the three domains. Furthermore, PICOT, Trx-HD, and Grx-HD possess an overall similar globular topology [6]. Each of the three domains possesses the canonical 'thioredoxin fold domain', formed by a central mix of 4 or 5 strand β-sheets flanked by three or more α-helices on either side of the β-strands [7, 8] (see Fig. **3**). The results imply that formation of the three domains during evolution originated from a single common ancestral gene. It should be mentioned however that several genes encode short proteins, which are built almost entirely of a PICOT-HD. It appears therefore that PICOT-HD-containing proteins may have biological functions that are independent of Trx or Grx, or that can operate in conjunction with other molecules, such as Trx or Grx.

INVOLVEMENT OF PICOT IN SIGNAL TRANSDUCTION

The original discovery of PICOT was based on its ability to interact with PKCθ [1], a critical enzyme for T cell antigen receptor (TCR)-linked signal transduction pathway [10, 11]. PKCθ is a Ca^{2+}-independent PKC isoform that plays an essential role in reorganization and/or function of the immunological synapse [12, 13]. It cooperates with calcineurin in activation of the interleukin-2 (IL-2) gene [3], and is involved in the promotion of signaling pathways that regulate T cell activation and survival [14-16]. Initial studies demonstrated that PICOT can serve as a negative regulator of PKCθ in T cells, since its overexpression resulted in downregulation of PKCθ-dependent activation of JNK, concomitantly with inhibition of the transcription factors AP-1 and NF-κB [1].

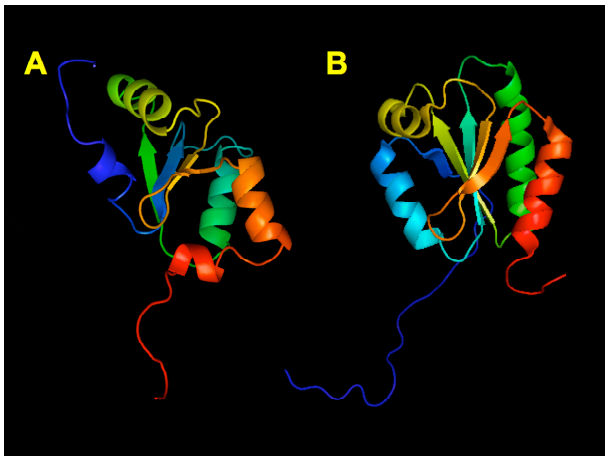

Fig (3). The 3D structure of the mouse PICOT-HD2 (**A**) and the human PICOT Trx-HD (**B**) were solved by K. Miyamoto *et al.* (PDB, 1WIK) and N. Tochio *et al.* (PDB, 2DIY), respectively. The figures were prepared using the PyMol Molecular Graphics System [9].

Physiological activation of T cells is initiated by TCR interaction with a major histocompatibility complex (MHC)-bound peptide antigen on the surface of antigen presenting cells (APC). The very early phase of the TCR-linked signal transduction pathway is regulated by protein tyrosine kinases (PTKs), which phosphorylate the cytoplasmic tails of the TCR associated CD3 subunits, as well as a plethora of effector molecules that are involved in signal transduction and induction of the activation response. Many of the effects induced by a physiological activation of tyrosine kinases can be mimicked by cell treatment with reactive oxygen intermediates (ROS), such as hydrogen peroxide, that were recently found to be produced *in vivo* and serve as physiological regulators of lymphocyte activation [17]. Other cell types were also found to produce ROS in response to cytokines and growth factors [18, 19]. One of the major sources for inducible ROS in leukocytes is the NADPH oxidase, which can be activated by several mechanisms, including phosphorylation by PKC [20].

The fact that PICOT associates with PKCθ and possesses Trx- and Grx-like sequences suggested that it might be involved in redox-regulated biochemical pathways. Treatment of Jurkat T cells with hydrogen peroxide resulted in phosphorylation of PICOT on tyrosine residues, an effect that could be abolished by inhibitors of Src family members of PTKs [21]. Furthermore, tyrosine phosphorylation of PICOT

was also observed in untreated cells following their transfection with a constitutively active Lck (Lck Y505F). The results suggest that tyrosine phosphorylation of PICOT can be directly or indirectly mediated by Lck. They also imply that PICOT plays a role in cell-activation dependent signaling pathways, and/or responses to stress signals mediated by reactive oxygen intermediates.

The fact that PKCθ is expressed predominantly in hematopoietic and muscle cells, in contrast to PICOT, which is expressed in a wide range of tissues, suggests that PICOT is likely to be involved in biological functions that are independent of PKCθ.

THE ROLE OF PICOT IN THE REGULATION OF HEART MUSCLE FUNCTION

The potential involvement of PICOT in cellular processes regulating heart muscle function was demonstrated by Jeong *et al.* during studies of the mechanisms leading to cardiac hypertrophy [4, 5]. These studies were aimed at the identification of potential regulators of cardiac hypertrophy, and led to the observation that PICOT was among several genes undergoing upregulation following transverse aortic constriction-induced cardiac hypertrophy of adult rat hearts. Furthermore, PICOT overexpression in the heart of transgenic mice inhibited cardiac hypertrophy induced by pressure overload, concomitantly with increase in ventricular function and cardiomyocyte contractility.

Jeong *et al.* have further analyzed the potential binding partners of PICOT in the muscle tissue, and using a GST-PICOT pull-down assay in conjunction with mass spectrometry they found that PICOT can directly interact with the muscle LIM domain protein (MLP) [4, 5], a member of a family of cysteine-rich proteins that mediate protein-protein interactions [22]. MLP is known to interact with several different proteins, including α-actinin, zyxin and calcineurin, all of which are known to concentrate at the Z-disc of the sarcomer [23, 24]. Immunofluorescence staining of tissue section of adult mouse heart using PICOT-specific Abs demonstrated PICOT localization within the Z-disc [5], results that were confirmed by our own studies (see Fig. **4**). In addition, PICOT was found to co-localize with MLP and another known Z-disc protein, the α-actinin [5]. MLP interaction with calcineurin, a Ca^{2+}-dependent Ser/Thr phosphatase, is known to be essential for calcineurin anchorage to the Z-disk and upregulation of its catalytic activity. The active, calcineurin then dephosphorylates the cytoplasmic NF-AT transcription factor and promotes its translocation to the nucleus where it induces the transcription of NF-AT regulated genes [23]. Overexpression of PICOT and its association with MLP interfered with MLP binding to calcineurin, causing displacement of calcineurin from its anchorage site at the Z disc. As a result, PICOT inhibited calcineurin-mediated dephosphorylation and subsequent nuclear translocation of NF-AT, as well as the NF-AT-regulated gene transcription. The authors suggest that PICOT inhibits cardiac hypertrophy by negative regulation of the MLP-calcineurin-NF-AT signaling pathway *via* a mechanism, which disrupts MLP-calcineurin interaction.

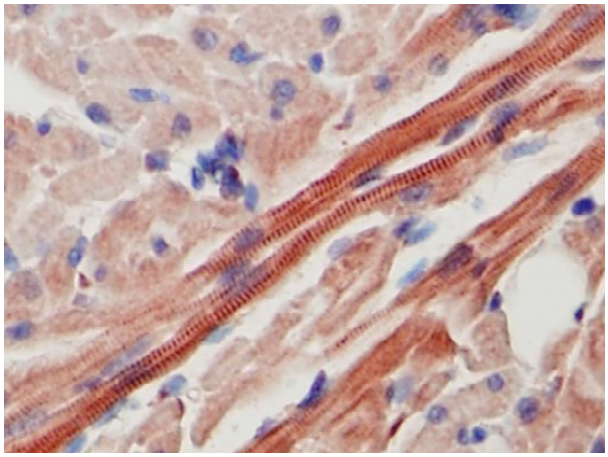

Fig. (4). Immunohistochemical staining of adult mouse heart sections with anti-PICOT Abs. Paraffin embedded sections of adult mouse heart were deparaffinized, mounted on glass slides, blocked with BSA, treated with hydrogen peroxide, and stained with rabbit anti-PICOT Abs plus biotinylated goat anti-rabbit IgG and peroxidase-conjugated streptavidin. Peroxidase activity was demonstrated by incubation with APC and hydrogen peroxide. Slides were counterstained with Hematoxylin and examined under a bright field microscope (Olympus I-70) attached to a digital camera (Olympus DP70).

POTENTIAL ROLE FOR PICOT DURING EMBRYOINC DEVELOPMENT

Comparative analysis of protein profiles expressed at different stages of embryonic development serves as a useful tool for the identification of molecules that are involved in specific developmental processes. In order to determine protein profile changes during embryogenesis Greene *et al.* [25] have utilized an immobilized pH gradient-based two-dimensional electrophoresis and identified individual protein spots by mass spectrometry. Analysis of proteins expressed between days (E) 8.5-to-10.5 of embryogenesis, the time of formation of the neural tube, revealed a number of changes in protein patterns at successive embryonic days. PICOT was almost undetectable at E8.5 but was dramatically upregulated at E9.5. Although the onset of PICOT expression correlated with that of PKCθ [26], the incomplete overlap in tissue expression of the two proteins suggest that PICOT may have developmental roles that are independent of PKCθ.

CONCLUSIONS

Despite the fact that the exact biological activity of PICOT is not yet known, indirect studies suggest that PICOT plays important roles in the regulation of T lymphocyte activation and during cardiac muscle responses to hypertrophy-inducing signals. PICOT is also predicted to be involved in certain developmental stages in mouse embryos, although the exact role or the tissue in which it is first expressed are not know. PICOT was shown to associate with PKCθ in T cells, but is also expressed in PKCθ-negative cell types indicating that PICOT assumes activities independent of PKCθ. Further studies of this novel protein and characterization of its exact biological activity may reveal important clues about

PICOT and the biological processes that are mediated by or dependent on PICOT.

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