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Contents

Continuous Volume 10 Number 1 May 27, 2020

EDITORIAL

1 PICOT promotes T lymphocyte proliferation by down-regulating cyclin D2 expression
Pandya P, Isakov N

Contents

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EDITORIAL

PICOT promotes T lymphocyte proliferation by down-regulating cyclin D2 expression

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Abstract

The mammalian protein kinase C-interacting cousin of thioredoxin (PICOT; also termed glutaredoxin 3) is a multi-domain monothiol glutaredoxin that is involved in a wide variety of signaling pathways and biological processes. PICOT is required for normal and transformed cell growth and is critical for embryonic development. Recent studies in T lymphocytes demonstrated that PICOT can translocate to the nucleus and interact with embryonic ectoderm development, a polycomb group protein and a core component of the polycomb repressive complex 2, which contributes to the maintenance of transcriptional repression and chromatin remodeling. Furthermore, PICOT was found to interact with chromatin-bound embryonic ectoderm development and alter the extent of histone 3 lysine 27 trimethylation at the promoter region of selected polycomb repressive complex 2 target genes. *PICOT* knockdown in Jurkat T cells led to increased histone 3 lysine 27 trimethylation at the promoter region of *CCND2*, a cell cycle-regulating gene which encodes the cyclin D2 protein. As a result, the expression levels of *CCND2* mRNA and protein levels were reduced, concomitantly with inhibition of the cell growth rate. Analysis of multiple data sets from the Cancer Genome Atlas revealed that a high expression of *PICOT* correlated with a low expression of *CCND2* in a large number of human cancers. In addition, this parameter correlated with poor patient survival, suggesting that the ratio between *PICOT*/*CCND2* mRNA levels might serve as a predictor of patient survival in selected types of human cancer.

Key words: Protein kinase C-interacting cousin of thioredoxin; Glutaredoxin 3; Cyclin D2; Histone methylation; T lymphocyte; Histone 3 lysine 27 trimethylation

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Core tip: Protein kinase C-interacting cousin of thioredoxin (PICOT) is a cell growth-regulating protein that interacts with embryonic ectoderm development, a core component of the polycomb repressive complex 2. PICOT is able to alter the extent of the tri-methylation of histone 3 lysine 27 at the promoter region of the polycomb repressive complex 2 target gene, *CCND2*, which encodes the cell cycle-regulating protein, cyclin D2. High expression levels of *PICOT* correlates with low expression levels of *CCND2* and poor survival of a large number of human cancer patients. The results suggest that a high *PICOT/CCND2* expression level ratio might serve as a predictor of patient survival in selected types of human cancer.

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INTRODUCTION

The discovery of protein kinase C-interacting cousin of thioredoxin (PICOT) was an unexpected off-shoot of studies of protein kinase C theta (PKCθ) and the attempts to define PKCθ-binding partners which regulate its behavior in T lymphocytes. PKCθ is a serine/threonine kinase which is highly expressed in T lymphocytes where it plays a critical role in signal transduction downstream of the T cell antigen receptor and contributes to the regulation of T lymphocyte activation, proliferation, differentiation and survival[1-3].

In order to identify new proteins that associate with PKCθ and might regulate its activity, a yeast two-hybrid screen was employed using the catalytically inactive PKCθ as a bait. A majority of the resultant colonies were found to encode a gene that its putative protein product contains a thioredoxin (Trx)-homologous domain. This putative protein was therefore termed PICOT, an acronym for PKC-interacting cousin of Trx[4]. Additional studies confirmed the expression of PICOT in mouse and human T-lymphocytes and its ability to interact with PKCθ and modulate PKCθ-dependent biochemical events[1]. The PICOT protein, also termed glutaredoxin 3 (Grx3), has a modular structure that includes three evolutionary conserved domains: A single N-terminal Trx homology domain and two PICOT/glutaredoxin 3 homology domains at its C-terminus[5,6]. In contrast to the classical Trx and Grx enzymes, which possess two redox-active cysteines at their catalytic site, PICOT possesses a single cysteine residue in the putative catalytic region of each of the three domains and is therefore devoid of the classical Trx and Grx catalytic activities. While the exact biological role of PICOT is not yet clear, PICOT appears to be critical for embryonic development since its knock-down in mice resulted in growth retardation and morphological changes, leading to death at approximately 12.5 d post coitum[7,8].

THE ROLE OF PICOT IN CELL GROWTH REGULATION

Initial attempts to identify the potential relationships between PICOT and PKCθ were performed in Jurkat T-lymphocytes that over-express a constitutively active PKCθ. Transient over-expression of PICOT in these cells led to down-regulation of the c-Jun N-terminal kinase (JNK) activity[1]. In addition, over-expressed PICOT inhibited JNK activity that was induced by co-transfected, constitutively-active PKCθ and calcineurin, a phosphatase that cooperates with PKCθ in the induction of JNK in antigen-triggered T-lymphocytes[1].

The expression levels of PICOT in highly proliferating T cell leukemia and lymphoma cell lines were found to be significantly higher than in freshly isolated T lymphocytes[9], while mitogenic activation of mouse splenic T-lymphocytes induced a concomitant increase in PICOT expression and cell proliferation[9], revealing a positive correlation between the expression level of PICOT and the rate of T proliferation. A more direct role for PICOT in cell growth regulation was demonstrated in several different cell types, including Jurkat T cells, cervical cancer-derived HeLa cells, breast cancer cells and mouse fibroblasts, where partial or complete knockdown of *PICOT* led to cell growth retardation[8,10,11].

Despite the fact that PICOT was discovered based on its ability to interact with PKCθ, histological, biochemical and molecular analyses demonstrated that PICOT was manifested in all PKCθ-expressing and PKCθ-non-expressing tissues and cell lines tested[8,9,12], suggesting that certain biological activities of PICOT are independent of PKCθ.

PICOT knockdown in HeLa cells resulted in a delayed mitotic exit and a higher percentage of binucleated cells, while PICOT-deficient mouse embryonic fibroblasts were found to accumulate at the G2/M phase due to cell cycle impairment[8]. Continuous efforts in our laboratory to obtain *PICOT* knock-out Jurkat T cells resuted in cell death within a few days after transfection and selection (unpublished). Mouse embryonic fibroblasts from PICOT-deficient cells did not survive *in vitro* beyond passage four[8] (and our own unpublished data). Our attempts to generate induced pluripotent stem cells from fibroblasts of PICOT-deficient mouse embryos by ectopic expression of *OCT4*, *SOX2*, *KLF4* and *MYC* (*OSKM*) vectors[13], using different protocols and a variety of culture conditions, were all unsuccessful (unpublished data).

Based on the above information we predict that PICOT is *sine qua non* for cell growth and survival and since the *PICOT* gene (*GLRX3*) has no functional redundant ortholog in mammals, its absence cannot be compensated for by other proteins.

PICOT ATTENUATES THE REACTIVE OXYGEN SPECIES-INDUCED DNA-DAMAGE RESPONSE

Reactive oxygen species (ROS) are generated as byproducts of the normal cellular metabolism and in response to environmental stimuli[14,15]. They include molecules such as superoxide, hydrogen peroxide and hydroxyl ions. Under normal physiological conditions, they function as second messengers in signal transduction pathways, and are therefore vital for developmental and growth processes[14,16-18]. Increased production of ROS due to stress signals is regarded as a host-defense mechanism which helps destroy exogenous pathogens[19]. However, due to the cytotoxic and reactive nature of ROS, excessive ROS production may damage macromolecules such as DNA, and induce single and double strand breaks which can cause cell cycle arrest, tissue destruction and cell death[20-23].

To overcome the oxidative damage, cells have developed an elaborate antioxidant network, in which glutaredoxins are major players[24]. A growing body of evidence suggests that monothiol glutaredoxins, such as PICOT, participate in the cellular mechanisms that regulate anti-stress responses.

Studies in Jurkat T cells demonstrated that in response to hydrogen peroxide, PICOT translocated to the nucleus where it underwent phosphorylation by tyrosine kinases[25]. In addition, PICOT-deficient Jurkat T cells and squamous cell carcinoma cells responded to stress conditions by a rise in the intracellular levels of ROS[26,27], and increase in the extent of the DNA fragmentation and the rate of cell mortality[26].

Overexpression of PICOT in cardiomyocytes protected the cells from ROS-induced damage triggered by myocardial ischemia-reperfusion injury, while infarction and apoptosis were exacerbated in the cardiac tissue of PICOT-deficient mice[28]. Moreover, C2C12 myoblasts responded to stress conditions by upregulating the PICOT mRNA[28].

Further studies demonstrated that PICOT-deficient Jurkat T cells responded to stress by decreased phosphorylation of the histone protein, H2AX, simultaneously with a decrease in cell survival. Phosphorylation of H2AX (then called γH2AX) is an early event that characterizes double-strand breaks in the DNA and is critical for the maintenance of genome stability[8]. The γH2AX promotes the assembly of DNA repair proteins at the site of damage and regulates the activation of checkpoint proteins, such as ataxia telangiectasia and Rad3-related protein (ATR) and its downstream targets, checkpoint kinase 1 and checkpoint kinase 2, which are essential for the DNA damage repair mechanism[8].

Stress conditions also promote the colocalization of PICOT with the DNA double strand breaks at the γH2AX-containing foci, as was demonstrated by immuno-fluorescent staining and confocal microscopy. In accord, PICOT-deficient cells responded to stress by a much slower rate of γH2AX foci formation. Furthermore, they reacted to stress by decreased phosphorylation and activation of ATR and its downstream kinases, which also regulate the γH2AX. It is therefore suggested that PICOT contributes to the cellular anti-stress response by promoting the ATR-dependent signaling pathways and activation of the DNA damage repair mechanism.

PICOT INTERACTS WITH THE CHROMATIN-ASSOCIATED EMBRYONIC ECTODERM DEVELOPMENT

The activity of a protein is often tied to the biological function of its binding partners. Many proteins function as components of large multiprotein complexes, in which the individual molecules cooperate with each other in order to carry out coordinated biological activities. To gain insight into the biological role of PICOT within T cells, a search for PICOT binding partners was carried out using a yeast two-hybrid system, which permits the identification of new protein interaction partners based on their physical association with a protein of interest. In this assay, a full-length human *PICOT* cDNA fused to the DNA binding domain of LexA was used as a bait for screening of a Jurkat T cell lymphoma cDNA library[29]. The results revealed that PICOT interacts with embryonic ectoderm development (EED)[30], a polycomb group protein that serves as a core component of the polycomb repressive complex 2 (PRC2) and contributes to the maintenance of transcriptional repression and chromatin remodeling and to the proliferation and differentiation of hematopoietic cells[31,32].

PICOT interaction with EED also occurs in Jurkat T cells as demonstrated by a reciprocal pull-down assay, using bead immobilized GST-PICOT and GST-EED fusion proteins[30]. Furthermore, immunofluorescent staining of Jurkat T cells demonstrated that PICOT and EED can colocalize, predominantly in the cell nuclei[30]. Pull-down assays using GST-EED-immobilized beads and lysates of Jurkat T cells expressing different Myc-tagged truncation products of PICOT, demonstrated that binding of EED is mediated by the two C-terminal PICOT homology domains, and that simultaneous interaction *via* both domains increases PICOT affinity for EED[30]. The ability of PICOT to associate with EED, which is a nuclear protein that contributes to the epigenetic regulation of gene transcription, suggest that PICOT might have nuclear functions that are related to the PRC2-mediated maintenance of transcriptional repression.

EED is an integral component of PRC2 and its major biological function is connected to the PRC2 complex-mediated histone methyltransferase activity, which promotes epigenetic gene silencing[32]. EED protein plays a critical role during embryonic development and its absence in mice results in death within 12.5 d post implantation[33-35].

The EED protein is one of four major core components of the PRC2 complex which also includes the zeste homolog 1 or 2 (EZH1/2), suppressor of zeste12 (SUZ12) and retinoblastoma protein-associated protein 46/48[32].

EZH1 and EZH2 are homologous proteins that serve as the PRC2 catalytic subunits that mediate the methyltransferase activity. While both proteins promote transcriptional silencing, they maintain repressive chromatin through different mechanisms[36,37]. EZH2 is the major isoform in Jurkat T cells which reside in the nucleus[38,39], and its expression is associated with cell proliferation[36]. In contrast, the expression levels of EZH1 in Jurkat T cells is very low, predominantly in the cytoplasm, where it can interact with ZAP70[40]. EZH2 requires both EED and SUZ12 in order to maintain the histone methyltransferase activity and the silencing function of the PRC2 complex[41,42].

The mammalian EED is present in cells as four distinct isoforms that are produced by alternative utilization of four different in-frame translation start sites in a common EED mRNA[43,44]. Usage of the EED isoforms is regulated developmentally, suggesting that PRC2 complexes containing distinct EED isoforms may differ in their histone methyltransferase activity and/or target selectivity[43]. Differential association of PICOT with selected EED isoforms may thus have distinct effects on the activity and specificity of the PRC2-mediated trimethylation of lysine 27 on histone 3 (H3K27).

The assembly and composition of PRC2 complexes are highly complicated because of the alternative usage of several different homologous core components, and the potential interaction of PRC2 with distinct auxiliary proteins[45,46]. As a result, the biological activity of the PRC2 complex might differ in accordance with the specific composition of its components.

Recent studies demonstrated that PRC2 complexes can be assembled by two alternative sets of proteins which give rise to two structurally and functionally distinct PRC2 complexes, termed PRC2.1 and PRC2.2. These two complexes exhibit antagonistic activities and regulate repression of different sets of polycomb target genes[47]. While the ability of PICOT to associate with either PRC2.1 or PRC2.2 has not yet been determined, the differential interaction of PICOT with PRC2.1 or PRC2.2 may have a different impact on the cellular epigenetic mechanisms that alter distinct cell-specific gene expression signatures (Figure 1). The PICOT-dependent differential regulation of gene expression may also be affected by the content of the EZH1/EZH2 catalytic subunit in PRC2, since presence of EZH1 or EZH2 in PRC2 is mutually

exclusive, and expression of the two catalytic subunits is subjected to distinct regulatory mechanisms. Furthermore, the existence of four distinct EED isoforms with which PICOT may associate, further increases the complexity and heterogeneity of this epigenetic mechanism.

PICOT REGULATION OF THE *CCND2* GENE EXPRESSION

The observation that PICOT interacts with EED provided the first indication that PICOT possibly plays a role in the regulation of PRC2-mediated trimethylation of histone H3 and contributes to the transcriptional and translational regulation of selected PRC2 target genes. To test whether this hypothesis is reliable, the ability of PICOT to interact with chromatin associated EED was analyzed using a chromatin immunoprecipitation (ChIP) assay. In this experiment, chromatin was immunoprecipitated from lysates of several different cell lines followed by sample fractionation on polyacrylamide gels and immunoblotting with anti-EED and anti-PICOT antibodies (Abs). The results indicated that chromatin fractions from all tested cell lines contained both EED and PICOT proteins[10]. PICOT and EED association with chromatin was verified in an heterologous system whereby chromatin was immunoprecipitated from samples of HA-PICOT and FLAG-EED transfected COS-7 cells. Using anti-HA and anti-FLAG Abs, the presence of the two heterologous proteins was observed in the chromatin fraction[10]. These results strengthened the assumption that nuclear PICOT might have a functional role in chromatin regulation.

Additional studies demonstrated that anti-PICOT Abs can coimmunoprecipitate chromatin-associated EED from Jurkat T cell-derived chromatin samples, but failed to coimmunoprecipitate other PRC2 core components, including EZH2 or SUZ12. Under similar assay conditions, anti-EED-Abs coimmunoprecipitated both PICOT and EZH2, suggesting that chromatin-associated EED can interact independently with either PICOT or EZH2, while PICOT binds chromatin-associated EED which is free of other PRC2 core components[10].

To further investigate whether PICOT association with chromatin-bound EED can alter the PRC2-mediated H3K27me3 levels at PRC2 target genes, a ChIP-quantitative polymerase chain reaction (qPCR) analysis was performed and the extent of H3K27me3 in chromatin lysates of PICOT-deficient *vs* wild-type Jurkat T cells was determined.

Initial screening indicated that *PICOT* knockdown had no effect on the global levels of H3K27me3. Similar findings were reported for other PRC2 auxiliary proteins, such as Jumonji and AT-rich interaction domain containing 2[10]. However, *PICOT* knockdown induced a significant decrease in the H3K27me3 mark at the PRC2 target genes, *CCND2*[10] and *Myt1*[30]. This was not an overall effect, since the extent of the H3K27me3 found at other PRC2 target genes, such as *HOXA2* and *ATF3*, was not altered[10].

The observation that *PICOT* knockdown led to a preferential desilencing of selected PRC2 target genes is not an exception, given that deletion of other PRC2 auxiliary proteins, such as the Polycomb-like proteins, led to similar effects[49].

The trimethylation of H3K27 is known to be catalyzed by EZH2[43,49] and further propagated by the help of EED[41,50]. To analyze whether the extent of these two proteins at the *CCND2* gene promoter is affected by PICOT, a ChIP-qPCR analysis was performed on chromatin lysates from wild-type and PICOT-deficient Jurkat T cells, using EZH2- and EED-specific Abs. The findings indicated that PICOT deficiency resulted in a signficant reduction in the extent of the EZH2 and EED at the *CCND2* gene promoter, suggesting that the effect of PICOT on H3K27me3 is mediated, at least in part, by promoting the recruitment of EZH2 and EED to this promoter region.

PRC2-mediated H3K27me3 has long been known to serve as a general mechanism for gene silencing[51,52]. Since *PICOT* knock down was found to decrease H3K27me3 at the *CCND2* gene promoter, an obvious question was whether this effect alters *CCND2* gene transcription. A qPCR analysis of the expression levels of *CCDN2* revealed that PICOT deficiency in Jurkat T cells led to a significant increase in *CCND2* mRNA levels, and a concomitant increase in expression of the *CCND2*-encoding protein, cyclin D2[10].

Thus, PICOT expression positively correlates with the extent of H3K27me3 at the *CCND2* gene promoter and negatively correlates with the *CCND2* mRNA and protein expression levels.

The cyclin D2 is a member of the D-type cyclin family of proteins which are implicated in cell cycle regulation, differentiation, and oncogenic transformation[53-55]. Increased expression of cyclin D2 was reported in normal primary cells and

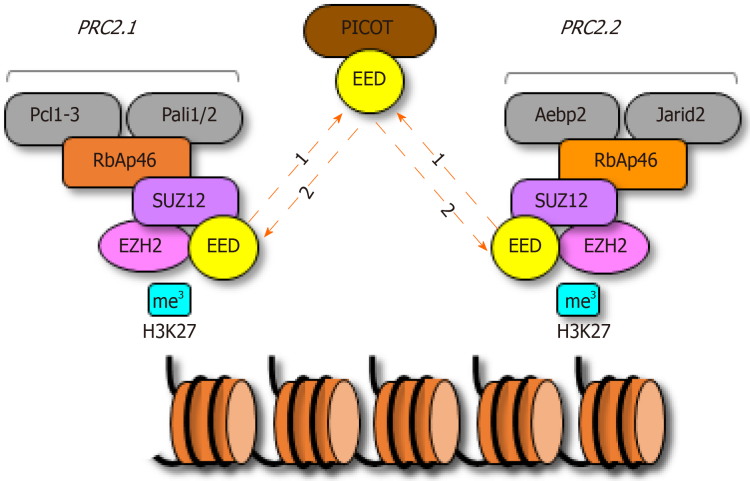


Figure 1 A schematic model showing the two distinct polycomb repressive complex 2 complexes and the potential alteration of their transcriptional repression activity by protein kinase C-interacting protein cousin of thioredoxin interaction with embryonic ectoderm development. Each of the two complexes possesses the same four core subunits, including embryonic ectoderm development (EED), suppressor of zeste12, RbAp46 (or RbAp48) and enhancer of zeste homolog 2 or 1 (EZH2 or EZH1), and is responsible for the mono-, di-, and tri-methylation of lysine 27 on histone H3 (H3K27me1/2/3) on chromatin. The polycomb repressive complex 2.1 (PRC2.1) complex is defined by its association with the Pcl1-3 and Pali1/2 auxiliary proteins, while PRC2.2 associates with Aebp2 and Jarid2. The two antagonistic PRC2 complexes exhibit divergent methyltransferase activities, they regulate the repression of different sets of polycomb target genes, and the balance between them is essential for the proper regulation of gene transcription. Recent findings demonstrated that protein kinase C-interacting protein cousin of thioredoxin (PICOT) association with EED can alter the transcription of certain PRC2 target genes, suggesting the involvement of PICOT in PRC2-dependent transcriptional regulation. A differential interaction of PICOT with PRC2.1- or PRC2.2-associated EED might have different impacts on cellular epigenetic mechanisms that alter distinct cell-specific gene expression signatures. Aebp2: Adipocyte enhancer-binding protein 2; EED: Embryonic ectoderm development; SUZ12: Suppressor of zeste 12; EZH2: Enhancer of zeste homolog 2; H3K27: Histone 3 lysine 27; Jarid2: Jumonji and AT-rich interaction domain containing 2; me^3: tri-methyl; Pali1: PRC2-associated LCOR isoform 1; Pcl1: Polycomb-like proteins 1; PICOT: Protein kinase C-interacting protein cousin of thioredoxin; PRC2: Polycomb repressive complex-2; RbAp46: Retinoblastoma protein-associated protein 46; SUZ12: Suppressor of Zeste 12.

established cell lines that were exposed to cell growth arrest-inducing conditions, such as contact inhibition, growth factor deprivation, and cellular senescence[56]. Further studies demonstrated that the nuclear cyclin D2 associates with, and activates cyclin-dependent kinases, such as CDK2 and CDK4[56,57] and promotes nuclear mechanisms leading to early embryonic cell cycle arrest[58].

We therefore hypothesize that the effect of PICOT on *CCND2* expression might alter the activity of cyclin D2 and its downstream target proteins which promote cell cycle arrest and cell growth retardation.

INVOLVEMENT OF *PICOT* AND *CCND2* IN TUMOR FORMATION

Various independent studies that analyzed diverse types of human cancer reported high expression levels of PICOT as a striking feature of the transformed cells. The tumors analyzed include Hodgkin's lymphoma[9], colorectal cancer[59], and carcinoma of the breast[11,12], lung[60,61], colon[60,62], and nasopharynx[63]. Overexpression of PICOT was also reported in human oral squamous cell carcinoma, where enhanced PICOT expression correlated with metastasis formation and decreased patient survival[27]. Furthermore, *PICOT* knockdown in oral squamous cell carcinoma-derived cell lines led to reduced Notch signaling concomitant with reversed epithelial-mesenchymal transition. These cells exhibited a significant reduction in *in vitro* migration and invasion, supporting a role for PICOT in tumor cell growth and dissemination.

Alterations in the expression levels of *CCND2* in uman cancers have also attracted considerable attention because of the critical role of cyclin D2 in cell cycle progression. Studies of malignant cells from lung[64], breast[65,66] and pancreatic cancer[67] revealed a significant reduction in the expression of the *CCND2* mRNA. These lower expression levels reflected hypermethylation of the *CCND2* gene promoter, which characterized

tumor cells, as it was rarely observed in normal cells that are adjacent to the tumor[64-67].

Studies performed in primary fibroblasts and established cell lines demonstrated that cell growth arrest resulted is characterized by a marked increase in cyclin D2 mRNA and protein expression[56], supporting the assumption that cyclin D2 functions as a negative regulator of cell growth.

This assumption was validated by several additional independent studies. For example, increased expression of cyclin D2 in prostate cancer cells led to inhibition of cell growth[68], while targeting of *CCND2* in non-small-cell lung carcinoma cells, using the miR-146a-5p microRNA, inhibited cell cycle progression and cell proliferation[69]. Additional, different types of *CCND2*-targeting miRs were found to downregulate the growth of prostate cancer cells, osteosarcoma, colorectal cancer, oral and laryngeal squamous cell carcinoma, glioblastoma, and gastric cancer[70-78]. The results suggest that *CCND2* expression levels are directly linked to cell growth regulation and that downregulation of *CCND2* is required for cell cycle progression and replication. It appears therefore that overexpressed PICOT in some types of tumor cells correlates with a hypermethylation of the *CCND2* gene promoter, which downregulates cyclin D2 expression levels, and in turn, promotes tumor cell growth and exacerbates tumor development.

MULTIPLE HUMAN CANCERS EXPRESS HIGH LEVELS OF PICOT AND LOW LEVELS OF *CCND2*

Based on the above observations and the findings showing that PICOT deficiency correlates with hypomethylation at the *CCND2* gene promoter and increased expression of cyclin D2, an in-depth analysis of the potential correlation between *PICOT* and *CCND2* mRNA expression levels in human cancers took place. This analysis was carried out on publicly available datasets from several thousand human cancers and 32 tumor types that were obtained from the Cancer Genome Atlas[10]. A general tendency of negative correlation between the expression levels of *PICOT* and *CCND2* mRNA was observed in most tumors, and a statistically significant negative correlation was shown in 8 out of the considered 32 tumor types considered. The strongest correlation was observed in prostate adenocarcinoma, lung adenocarcinoma (LUAD) and pancreatic adenocarcinoma (PAAD), with lower, but significant values in glioblastoma multiforme, breast invasive carcinoma (BRCA), gastric adenocarcinoma, esophageal carcinoma and ovarian cancer[1].

In contrast to the results showing a negative correlation between *PICOT* and *CCND2* mRNA expression levels observed in BRCA and LUAD, an opposite correlation was observed in normal breast and lung tissue cells which are adjacent to the tumor[1]. It appears therefore that the negative correlation between *PICOT* and *CCND2* mRNA levels in the cancer cells represents an abnormal characteristic of the BRCA and LUAD cancer cells, rather than a tissue-specific phenomenon. These findings also suggest that the *PICOT*/*CCND2* expression ratio might serve as a predictor of patient survival in selected human cancers. The observation that a negative correlation between *PICOT* and *CCND2* mRNA expression occurs in some but not all types of cancer may indicate that growth regulation of cancer cells is controlled by multiple genes, including tissue specific genes, which vary in their overall impact on the growth regulation of distinct types of cells.

HIGH EXPRESSION OF *PICOT* AND LOW EXPRESSION OF *CCND2* CORRELATES WITH POOR PATIENT SURVIVAL IN SEVERAL TYPES OF HUMAN CANCER

The negative correlation between PICOT and cyclin D2 expression levels in selected types of human cancer suggested that the ratio between the expression levels of PICOT cyclin D2 might be related to the progression rate of cancer diseases and may impact on the overall patients' survival.

To analyze this potential linkage, *PICOT* and *CCND2* mRNA levels and cancer patients' clinical data were obtained from the Cancer Genome Atlas. Patients from each of the five types of tumors selected were divided, based on their gene expression level, to high and low expressers. Kaplan-Meier survival plots for gene expression *vs* survival probability, a log-rank *P* value, and a hazard ratio were compared in each type of cancer. The data revealed a significant log-rank *P* value for high expression of *PICOT* in LUAD, PAAD, BRCA, and esophageal carcinoma and for low expression of

CCND2 in LUAD, PAAD, BRCA and glioblastoma multiforme. Survival probability of patients from four different types of cancer relative to the expression levels of *PICOT* and *CCND2* in the tumor cells is presented in Figure 2. The results suggest that overexpression of *PICOT* and underexpression of *CCND2* correlate with poor overall patient survival in the majority of the tumor types analyzed. Further substantiation of these findings may yield a useful predictor of patient survival in selected types of human cancer. In addition, they might help evaluate new anti-cancer drugs and determine their efficiency based on their ability to modulate the expression levels of *PICOT* and *CCND2*.

CONCLUDING REMARKS

PICOT is a ubiquitous protein that plays an important role in a variety of biochemical pathways and cellular processes. The observation that PICOT interacts with EED, an integral component of the PRC2 complex, suggests that PICOT has nuclear functions that might impact on transcriptional repression and chromatin remodeling. This hypothesis was further strengthened by studies in Jurkat T cells, in which a fraction of the cellular PICOT was found to reside in the nucleus, interact with chromatin-bound EED, and modulate trimethylation of H3K27 at selected PRC2 target genes, specifically, *CCND2*.

An increased expression of *PICOT* and a concomitant decrease in the expression of *CCND2* correlated with poor patient survival in several types of human cancers. The *PICOT/CCND2* expression ratio can therefore serve as a predictor of patient survival in certain types of cancer, and perhaps be used as a marker for therapeutic drug monitoring and assessment of effectiveness of drug doses. Further clinical and molecular sub-classification of tumor subtypes are required in order to validate the cancers in which the *PICOT/CCND2* expression ratio can serve as a reliable tumor cell marker. The subtyping of cancer can provide important biological insights into cancer heterogeneity and is likely to serve as a critical step for the application of individualized therapy. Accurate signature-based classifications of human tumors are currently in progress using advanced machine learning technologies which integrate multiple types of transcriptomic and proteomic data with deep learning approaches[79,80]. The subtyping of cancer will help define the molecular and genetic landscape of distinct types of human cancer[81,82], and define the cancer subtypes which are characterized by a high ratio of *PICOT/CCND2* expression levels that might serve as an accurate risk predictor.

Understanding the biological role of PICOT and identification of its structure and mechanism of interaction with EED and other binding partners will help design new drugs for pharmacological intervention with PICOT functions, help inhibit cell growth and serve as efficient tools for cancer therapy.

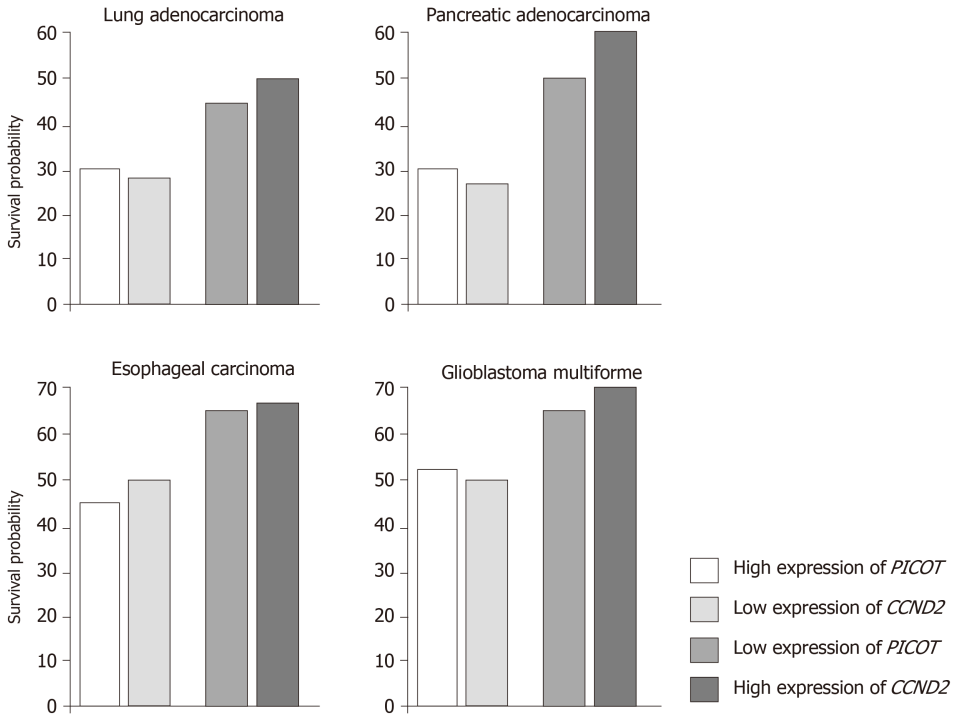


Figure 2 High expression of *PICOT* and low expression of *CCND2* correlate with poor patient survival in different types of human cancer. *PICOT* and *CCND2* mRNA expression and cancer patients' clinical data were derived from the Cancer Genome Atlas database. Patients were divided into high or low expression groups, using the maximally selected rank statistics that is implemented in the R package "survminer". Survival of patients was visualized using the Kaplan–Meier estimator and the 5 years survival probability of lung adenocarcinoma and 2 years survival probability of pancreatic adenocarcinoma, esophageal carcinoma and glioblastoma multiforme were calculated and presented in the bar graph.

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