**Name of Journal:** *World Journal of Immunology*

**Manuscript NO:** 54388

**Manuscript Type:** EDITORIAL

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

Pandya P *et al*. PICOT regulates *CCND2* gene expression

Pinakin Pandya, Noah Isakov

**Pinakin Pandya, Noah Isakov,** The Shraga Segal Department of Microbiology, Immunology and Genetics, Faculty of Health Sciences and the Cancer Research Center, Ben Gurion University of the Negev, Beer Sheva 84105, Israel

**Pinakin Pandya,** Department of Computational and System biology, UPMC Hillman Cancer Center, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15232, United States

**Author contributions:** This manuscript was written and edited by both authors.

**Supported by** the USA-Israel Binational Science Foundation, No. 2013034; the Israel Science Foundation administered by the Israel Academy of Science, No. 1235/17; the Jacki and Bruce Barron Cancer Research Scholars’ Program, City of Hope and the Israel Cancer Research Fund, No. 87735611.

**Corresponding author: Noah Isakov,PhD, Professor,** The Shraga Segal Department of Microbiology, Immunology and Genetics, Faculty of Health Sciences and the Cancer Research Center, Ben Gurion University of the Negev, P.O.B. 653, Beer Sheva 84105, Israel. noah@bgu.ac.il

**Received:** January 25, 2020

**Revised:** May 9, 2020

**Accepted:** May 12, 2020

**Published online:** May 27, 2020

**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

Pandya P, Isakov N. PICOT promotes T lymphocyte proliferation by down-regulating cyclin D2 expression. *World J Immunol* 2020; 10(1): 1-12 URL: https://www.wjgnet.com/2219-2824/full/v10/i1/1.htm DOI: https://dx.doi.org/10.5411/wji.v10.i1.1

**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.

**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** R**EACTIVE 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 immunofluorescent 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 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* mRNAexpression 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.

**ACKNOWLEDGEMENTS**

We thank Caroline Simon for editorial assistance.

**REFERENCES**

1 **Baier G**, Telford D, Giampa L, Coggeshall KM, Baier-Bitterlich G, Isakov N, Altman A. Molecular cloning and characterization of PKC theta, a novel member of the protein kinase C (PKC) gene family expressed predominantly in hematopoietic cells. *J Biol Chem* 1993; **268**: 4997-5004 [PMID: 8444877]

2 **Isakov N**, Altman A. Protein kinase C(theta) in T cell activation. *Annu Rev Immunol* 2002; **20**: 761-794 [PMID: 11861617 DOI: 10.1146/annurev.immunol.20.100301.064807]

3 **Zhang EY**, Kong KF, Altman A. The yin and yang of protein kinase C-theta (PKCθ): a novel drug target for selective immunosuppression. *Adv Pharmacol* 2013; **66**: 267-312 [PMID: 23433459 DOI: 10.1016/B978-0-12-404717-4.00006-8]

4 **Witte S**, Villalba M, Bi K, Liu Y, Isakov N, Altman A. Inhibition of the c-Jun N-terminal kinase/AP-1 and NF-kappaB pathways by PICOT, a novel protein kinase C-interacting protein with a thioredoxin homology domain. *J Biol Chem* 2000; **275**: 1902-1909 [PMID: 10636891 DOI: 10.1074/jbc.275.3.1902]

5 **Isakov N**, Witte S, Altman A. PICOT-HD: a highly conserved protein domain that is often associated with thioredoxin and glutaredoxin modules. *Trends Biochem Sci* 2000; **25**: 537-539 [PMID: 11084362 DOI: 10.1016/s0968-0004(00)01685-6]

6 **Babichev Y,** Witte S, Altman A, Isakov N. The human PICOT protein possesses a thioredoxin-like homology domain and a tandem repeat of a novel domain which is highly conserved during evolution. 2001. IOS Press OHM, Amsterdam, Berlin, Oxford, Tokyo Washington, DC

7 **Cha H**, Kim JM, Oh JG, Jeong MH, Park CS, Park J, Jeong HJ, Park BK, Lee YH, Jeong D, Yang DK, Bernecker OY, Kim DH, Hajjar RJ, Park WJ. PICOT is a critical regulator of cardiac hypertrophy and cardiomyocyte contractility. *J Mol Cell Cardiol* 2008; **45**: 796-803 [PMID: 18929570 DOI: 10.1016/j.yjmcc.2008.09.124]

8 **Cheng NH**, Zhang W, Chen WQ, Jin J, Cui X, Butte NF, Chan L, Hirschi KD. A mammalian monothiol glutaredoxin, Grx3, is critical for cell cycle progression during embryogenesis. *FEBS J* 2011; **278**: 2525-2539 [PMID: 21575136 DOI: 10.1111/j.1742-4658.2011.08178.x]

9 **Ohayon A**, Babichev Y, Pasvolsky R, Dong G, Sztarkier I, Benharroch D, Altman A, Isakov N. Hodgkin's lymphoma cells exhibit high expression levels of the PICOT protein. *J Immunotoxicol* 2010; **7**: 8-14 [PMID: 20170406 DOI: 10.3109/15476910903427654]

10 **Pandya P**, Jethva M, Rubin E, Birnbaum RY, Braiman A, Isakov N. PICOT binding to chromatin-associated EED negatively regulates cyclin D2 expression by increasing H3K27me3 at the CCND2 gene promoter. *Cell Death Dis* 2019; **10**: 685 [PMID: 31527584 DOI: 10.1038/s41419-019-1935-0]

11 **Qu Y**, Wang J, Ray PS, Guo H, Huang J, Shin-Sim M, Bukoye BA, Liu B, Lee AV, Lin X, Huang P, Martens JW, Giuliano AE, Zhang N, Cheng NH, Cui X. Thioredoxin-like 2 regulates human cancer cell growth and metastasis via redox homeostasis and NF-κB signaling. *J Clin Invest* 2011; **121**: 212-225 [PMID: 21123948 DOI: 10.1172/JCI43144]

12 **Ohayon A**, Babichev Y, Galperin M, Altman A, Isakov N. Widespread expression of PICOT in mouse and human tissues with predominant localization to epithelium. *J Histochem Cytochem* 2010; **58**: 799-806 [PMID: 20498481 DOI: 10.1369/jhc.2010.956532]

13 **Driscoll CB**, Tonne JM, El Khatib M, Cattaneo R, Ikeda Y, Devaux P. Nuclear reprogramming with a non-integrating human RNA virus. *Stem Cell Res Ther* 2015; **6**: 48 [PMID: 25889591 DOI: 10.1186/s13287-015-0035-z]

14 **Dröge W**. Free radicals in the physiological control of cell function. *Physiol Rev* 2002; **82**: 47-95 [PMID: 11773609 DOI: 10.1152/physrev.00018.2001]

15 **Finkel T**. Oxidant signals and oxidative stress. *Curr Opin Cell Biol* 2003; **15**: 247-254 [PMID: 12648682 DOI: 10.1016/s0955-0674(03)00002-4]

16 **Trachootham D**, Lu W, Ogasawara MA, Nilsa RD, Huang P. Redox regulation of cell survival. *Antioxid Redox Signal* 2008; **10**: 1343-1374 [PMID: 18522489 DOI: 10.1089/ars.2007.1957]

17 **Ray PD**, Huang BW, Tsuji Y. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cell Signal* 2012; **24**: 981-990 [PMID: 22286106 DOI: 10.1016/j.cellsig.2012.01.008]

18 **Dennery PA**. Effects of oxidative stress on embryonic development. *Birth Defects Res C Embryo Today* 2007; **81**: 155-162 [PMID: 17963268 DOI: 10.1002/bdrc.20098]

19 **Bergendi L**, Benes L, Duracková Z, Ferencik M. Chemistry, physiology and pathology of free radicals. *Life Sci* 1999; **65**: 1865-1874 [PMID: 10576429 DOI: 10.1016/s0024-3205(99)00439-7]

20 **Yahata T**, Takanashi T, Muguruma Y, Ibrahim AA, Matsuzawa H, Uno T, Sheng Y, Onizuka M, Ito M, Kato S, Ando K. Accumulation of oxidative DNA damage restricts the self-renewal capacity of human hematopoietic stem cells. *Blood* 2011; **118**: 2941-2950 [PMID: 21734240 DOI: 10.1182/blood-2011-01-330050]

21 **Hayashi T**, Hayashi I, Shinohara T, Morishita Y, Nagamura H, Kusunoki Y, Kyoizumi S, Seyama T, Nakachi K. Radiation-induced apoptosis of stem/progenitor cells in human umbilical cord blood is associated with alterations in reactive oxygen and intracellular pH. *Mutat Res* 2004; **556**: 83-91 [PMID: 15491635 DOI: 10.1016/j.mrfmmm.2004.07.002]

22 **Yoshida T**, Goto S, Kawakatsu M, Urata Y, Li TS. Mitochondrial dysfunction, a probable cause of persistent oxidative stress after exposure to ionizing radiation. *Free Radic Res* 2012; **46**: 147-153 [PMID: 22126415 DOI: 10.3109/10715762.2011.645207]

23 **Shackelford RE**, Kaufmann WK, Paules RS. Oxidative stress and cell cycle checkpoint function. *Free Radic Biol Med* 2000; **28**: 1387-1404 [PMID: 10924858 DOI: 10.1016/s0891-5849(00)00224-0]

24 **Lillig CH**, Berndt C, Holmgren A. Glutaredoxin systems. *Biochim Biophys Acta* 2008; **1780**: 1304-1317 [PMID: 18621099 DOI: 10.1016/j.bbagen.2008.06.003]

25 **Babichev Y**, Isakov N. Tyrosine phosphorylation of PICOT and its translocation to the nucleus in response of human T cells to oxidative stress. *Adv Exp Med Biol* 2001; **495**: 41-45 [PMID: 11774602 DOI: 10.1007/978-1-4615-0685-0\_6]

26 **Pandya P**, Braiman A, Isakov N. PICOT (GLRX3) is a positive regulator of stress-induced DNA-damage response. *Cell Signal* 2019; **62**: 109340 [PMID: 31176019 DOI: 10.1016/j.cellsig.2019.06.005]

27 **Li B**, Chen M, Lu M, Xin-Xiang J, Meng-Xiong P, Jun-Wu M. Glutaredoxin 3 promotes migration and invasion via the Notch signalling pathway in oral squamous cell carcinoma. *Free Radic Res* 2018; **52**: 390-401 [PMID: 29397791 DOI: 10.1080/10715762.2018.1435871]

28 **Kim J**, Kim J, Kook H, Park WJ. PICOT alleviates myocardial ischemia-reperfusion injury by reducing intracellular levels of reactive oxygen species. *Biochem Biophys Res Commun* 2017; **485**: 807-813 [PMID: 28257842 DOI: 10.1016/j.bbrc.2017.02.136]

29 **Witte S**, Krawinkel U. Specific interactions of the autoantigen L7 with multi-zinc finger protein ZNF7 and ribosomal protein S7. *J Biol Chem* 1997; **272**: 22243-22247 [PMID: 9268371 DOI: 10.1074/jbc.272.35.22243]

30 **Pandya P**, Pasvolsky R, Babichev Y, Braiman A, Witte S, Altman A, Isakov N. PICOT binding to the polycomb group protein, EED, alters H3K27 methylation at the MYT1 PRC2 target gene. *Biochem Biophys Res Commun* 2019; **509**: 469-475 [PMID: 30595380 DOI: 10.1016/j.bbrc.2018.12.153]

31 **Margueron R**, Reinberg D. The Polycomb complex PRC2 and its mark in life. *Nature* 2011; **469**: 343-349 [PMID: 21248841 DOI: 10.1038/nature09784]

32 **Cao Q**, Wang X, Zhao M, Yang R, Malik R, Qiao Y, Poliakov A, Yocum AK, Li Y, Chen W, Cao X, Jiang X, Dahiya A, Harris C, Feng FY, Kalantry S, Qin ZS, Dhanasekaran SM, Chinnaiyan AM. The central role of EED in the orchestration of polycomb group complexes. *Nat Commun* 2014; **5**: 3127 [PMID: 24457600 DOI: 10.1038/ncomms4127]

33 **Faust C**, Schumacher A, Holdener B, Magnuson T. The eed mutation disrupts anterior mesoderm production in mice. *Development* 1995; **121**: 273-285 [PMID: 7768172]

34 **Wang J**, Mager J, Schnedier E, Magnuson T. The mouse PcG gene eed is required for Hox gene repression and extraembryonic development. *Mamm Genome* 2002; **13**: 493-503 [PMID: 12370779 DOI: 10.1007/s00335-002-2182-7]

35 **Morin-Kensicki EM**, Faust C, LaMantia C, Magnuson T. Cell and tissue requirements for the gene eed during mouse gastrulation and organogenesis. *Genesis* 2001; **31**: 142-146 [PMID: 11783004 DOI: 10.1002/gene.10017]

36 **Margueron R**, Li G, Sarma K, Blais A, Zavadil J, Woodcock CL, Dynlacht BD, Reinberg D. Ezh1 and Ezh2 maintain repressive chromatin through different mechanisms. *Mol Cell* 2008; **32**: 503-518 [PMID: 19026781 DOI: 10.1016/j.molcel.2008.11.004]

37 **Denisenko ON**, Bomsztyk K. The product of the murine homolog of the Drosophila extra sex combs gene displays transcriptional repressor activity. *Mol Cell Biol* 1997; **17**: 4707-4717 [PMID: 9234727 DOI: 10.1128/mcb.17.8.4707]

38 **Denisenko O**, Shnyreva M, Suzuki H, Bomsztyk K. Point mutations in the WD40 domain of Eed block its interaction with Ezh2. *Mol Cell Biol* 1998; **18**: 5634-5642 [PMID: 9742080 DOI: 10.1128/mcb.18.10.5634]

39 **Su IH**, Dobenecker MW, Dickinson E, Oser M, Basavaraj A, Marqueron R, Viale A, Reinberg D, Wülfing C, Tarakhovsky A. Polycomb group protein ezh2 controls actin polymerization and cell signaling. *Cell* 2005; **121**: 425-436 [PMID: 15882624 DOI: 10.1016/j.cell.2005.02.029]

40 **Ogawa M**, Hiraoka Y, Aiso S. The Polycomb-group protein ENX-2 interacts with ZAP-70. *Immunol Lett* 2003; **86**: 57-61 [PMID: 12600746 DOI: 10.1016/s0165-2478(02)00293-6]

41 **Montgomery ND**, Yee D, Chen A, Kalantry S, Chamberlain SJ, Otte AP, Magnuson T. The murine polycomb group protein Eed is required for global histone H3 lysine-27 methylation. *Curr Biol* 2005; **15**: 942-947 [PMID: 15916951 DOI: 10.1016/j.cub.2005.04.051]

42 **Pasini D**, Bracken AP, Jensen MR, Lazzerini Denchi E, Helin K. Suz12 is essential for mouse development and for EZH2 histone methyltransferase activity. *EMBO J* 2004; **23**: 4061-4071 [PMID: 15385962 DOI: 10.1038/sj.emboj.7600402]

43 **Kuzmichev A**, Jenuwein T, Tempst P, Reinberg D. Different EZH2-containing complexes target methylation of histone H1 or nucleosomal histone H3. *Mol Cell* 2004; **14**: 183-193 [PMID: 15099518 DOI: 10.1016/s1097-2765(04)00185-6]

44 **Montgomery ND**, Yee D, Montgomery SA, Magnuson T. Molecular and functional mapping of EED motifs required for PRC2-dependent histone methylation. *J Mol Biol* 2007; **374**: 1145-1157 [PMID: 17997413 DOI: 10.1016/j.jmb.2007.10.040]

45 **Hauri S**, Comoglio F, Seimiya M, Gerstung M, Glatter T, Hansen K, Aebersold R, Paro R, Gstaiger M, Beisel C. A High-Density Map for Navigating the Human Polycomb Complexome. *Cell Rep* 2016; **17**: 583-595 [PMID: 27705803 DOI: 10.1016/j.celrep.2016.08.096]

46 **Smits AH**, Jansen PW, Poser I, Hyman AA, Vermeulen M. Stoichiometry of chromatin-associated protein complexes revealed by label-free quantitative mass spectrometry-based proteomics. *Nucleic Acids Res* 2013; **41**: e28 [PMID: 23066101 DOI: 10.1093/nar/gks941]

47 **Conway E**, Jerman E, Healy E, Ito S, Holoch D, Oliviero G, Deevy O, Glancy E, Fitzpatrick DJ, Mucha M, Watson A, Rice AM, Chammas P, Huang C, Pratt-Kelly I, Koseki Y, Nakayama M, Ishikura T, Streubel G, Wynne K, Hokamp K, McLysaght A, Ciferri C, Di Croce L, Cagney G, Margueron R, Koseki H, Bracken AP. A Family of Vertebrate-Specific Polycombs Encoded by the LCOR/LCORL Genes Balance PRC2 Subtype Activities. *Mol Cell* 2018; **70**: 408-421.e8 [PMID: 29628311 DOI: 10.1016/j.molcel.2018.03.005]

48 **Sanulli S**, Justin N, Teissandier A, Ancelin K, Portoso M, Caron M, Michaud A, Lombard B, da Rocha ST, Offer J, Loew D, Servant N, Wassef M, Burlina F, Gamblin SJ, Heard E, Margueron R. Jarid2 Methylation via the PRC2 Complex Regulates H3K27me3 Deposition during Cell Differentiation. *Mol Cell* 2015; **57**: 769-783 [PMID: 25620564 DOI: 10.1016/j.molcel.2014.12.020]

49 **Lanzuolo C**, Orlando V. Memories from the polycomb group proteins. *Annu Rev Genet* 2012; **46**: 561-589 [PMID: 22994356 DOI: 10.1146/annurev-genet-110711-155603]

50 **Margueron R**, Justin N, Ohno K, Sharpe ML, Son J, Drury WJ 3rd, Voigt P, Martin SR, Taylor WR, De Marco V, Pirrotta V, Reinberg D, Gamblin SJ. Role of the polycomb protein EED in the propagation of repressive histone marks. *Nature* 2009; **461**: 762-767 [PMID: 19767730 DOI: 10.1038/nature08398]

51 **Laugesen A**, Højfeldt JW, Helin K. Molecular Mechanisms Directing PRC2 Recruitment and H3K27 Methylation. *Mol Cell* 2019; **74**: 8-18 [PMID: 30951652 DOI: 10.1016/j.molcel.2019.03.011]

52 **Yu JR**, Lee CH, Oksuz O, Stafford JM, Reinberg D. PRC2 is high maintenance. *Genes Dev* 2019; **33**: 903-935 [PMID: 31123062 DOI: 10.1101/gad.325050.119]

53 **Robker RL**, Richards JS. Hormone-induced proliferation and differentiation of granulosa cells: a coordinated balance of the cell cycle regulators cyclin D2 and p27Kip1. *Mol Endocrinol* 1998; **12**: 924-940 [PMID: 9658398 DOI: 10.1210/mend.12.7.0138]

54 **Qian L**, Gong J, Liu J, Broome JD, Koduru PR. Cyclin D2 promoter disrupted by t(12;22)(p13;q11.2) during transformation of chronic lymphocytic leukaemia to non-Hodgkin's lymphoma. *Br J Haematol* 1999; **106**: 477-485 [PMID: 10460609 DOI: 10.1046/j.1365-2141.1999.01549.x]

55 **Russo LC**, Araujo CB, Iwai LK, Ferro ES, Forti FL. A Cyclin D2-derived peptide acts on specific cell cycle phases by activating ERK1/2 to cause the death of breast cancer cells. *J Proteomics* 2017; **151**: 24-32 [PMID: 27371349 DOI: 10.1016/j.jprot.2016.06.028]

56 **Meyyappan M**, Wong H, Hull C, Riabowol KT. Increased expression of cyclin D2 during multiple states of growth arrest in primary and established cells. *Mol Cell Biol* 1998; **18**: 3163-3172 [PMID: 9584157 DOI: 10.1128/mcb.18.6.3163]

57 **Sweeney KJ**, Sarcevic B, Sutherland RL, Musgrove EA. Cyclin D2 activates Cdk2 in preference to Cdk4 in human breast epithelial cells. *Oncogene* 1997; **14**: 1329-1340 [PMID: 9178893 DOI: 10.1038/sj.onc.1200951]

58 **Taieb F**, Chartrain I, Chevalier S, Haccard O, Jessus C. Cyclin D2 arrests Xenopus early embryonic cell cycles. *Exp Cell Res* 1997; **237**: 338-346 [PMID: 9434629 DOI: 10.1006/excr.1997.3800]

59 **Lu Y**, Wang X, Liu Z, Jin B, Chu D, Zhai H, Zhang F, Li K, Ren G, Miranda-Vizuete A, Guo X, Fan D. Identification and distribution of thioredoxin-like 2 as the antigen for the monoclonal antibody MC3 specific to colorectal cancer. *Proteomics* 2008; **8**: 2220-2229 [PMID: 18528843 DOI: 10.1002/pmic.200700770]

60 **Cha MK**, Kim IH. Preferential overexpression of glutaredoxin3 in human colon and lung carcinoma. *Cancer Epidemiol* 2009; **33**: 281-287 [PMID: 19797004 DOI: 10.1016/j.canep.2009.08.006]

61 **Zhu YY**, Wang ZJ, Ma N, Zhou JW. [Proliferation and apoptosis of lung cancer cells regulated by gultaredoxin 3]. *Zhonghua Zhong Liu Za Zhi* 2018; **40**: 325-329 [PMID: 29860757 DOI: 10.3760/cma.j.issn.0253-3766.2018.05.002]

62 **Lu Y**, Zhao X, Li K, Luo G, Nie Y, Shi Y, Zhou Y, Ren G, Feng B, Liu Z, Pan Y, Li T, Guo X, Wu K, Miranda-Vizuete A, Wang X, Fan D. Thioredoxin-like protein 2 is overexpressed in colon cancer and promotes cancer cell metastasis by interaction with ran. *Antioxid Redox Signal* 2013; **19**: 899-911 [PMID: 23311631 DOI: 10.1089/ars.2012.4736]

63 **He F**, Wei L, Luo W, Liao Z, Li B, Zhou X, Xiao X, You J, Chen Y, Zheng S, Li P, Murata M, Huang G, Zhang Z. Glutaredoxin 3 promotes nasopharyngeal carcinoma growth and metastasis via EGFR/Akt pathway and independent of ROS. *Oncotarget* 2016; **7**: 37000-37012 [PMID: 27203742 DOI: 10.18632/oncotarget.9454]

64 **Virmani A**, Rathi A, Heda S, Sugio K, Lewis C, Tonk V, Takahashi T, Roth JA, Minna JD, Euhus DM, Gazdar AF. Aberrant methylation of the cyclin D2 promoter in primary small cell, nonsmall cell lung and breast cancers. *Int J Cancer* 2003; **107**: 341-345 [PMID: 14506731 DOI: 10.1002/ijc.11393]

65 **Evron E**, Umbricht CB, Korz D, Raman V, Loeb DM, Niranjan B, Buluwela L, Weitzman SA, Marks J, Sukumar S. Loss of cyclin D2 expression in the majority of breast cancers is associated with promoter hypermethylation. *Cancer Res* 2001; **61**: 2782-2787 [PMID: 11289162]

66 **Buckley MF**, Sweeney KJ, Hamilton JA, Sini RL, Manning DL, Nicholson RI, deFazio A, Watts CK, Musgrove EA, Sutherland RL. Expression and amplification of cyclin genes in human breast cancer. *Oncogene* 1993; **8**: 2127-2133 [PMID: 8336939]

67 **Matsubayashi H**, Sato N, Fukushima N, Yeo CJ, Walter KM, Brune K, Sahin F, Hruban RH, Goggins M. Methylation of cyclin D2 is observed frequently in pancreatic cancer but is also an age-related phenomenon in gastrointestinal tissues. *Clin Cancer Res* 2003; **9**: 1446-1452 [PMID: 12684418]

68 **Witt D**, Burfeind P, von Hardenberg S, Opitz L, Salinas-Riester G, Bremmer F, Schweyer S, Thelen P, Neesen J, Kaulfuss S. Valproic acid inhibits the proliferation of cancer cells by re-expressing cyclin D2. *Carcinogenesis* 2013; **34**: 1115-1124 [PMID: 23349020 DOI: 10.1093/carcin/bgt019]

69 **Li YL**, Wang J, Zhang CY, Shen YQ, Wang HM, Ding L, Gu YC, Lou JT, Zhao XT, Ma ZL, Jin YX. MiR-146a-5p inhibits cell proliferation and cell cycle progression in NSCLC cell lines by targeting CCND1 and CCND2. *Oncotarget* 2016; **7**: 59287-59298 [PMID: 27494902 DOI: 10.18632/oncotarget.11040]

70 **Zhu C**, Shao P, Bao M, Li P, Zhou H, Cai H, Cao Q, Tao L, Meng X, Ju X, Qin C, Li J, Yin C. miR-154 inhibits prostate cancer cell proliferation by targeting CCND2. *Urol Oncol* 2014; **32**: 31.e9-31.16 [PMID: 23428540 DOI: 10.1016/j.urolonc.2012.11.013]

71 **Zhang F**, Zhu Y, Fan G, Hu S. *MicroRNA-2682-3p* inhibits osteosarcoma cell proliferation by targeting *CCND2, MMP8* and *Myd88*. *Oncol Lett* 2018; **16**: 3359-3364 [PMID: 30127935 DOI: 10.3892/ol.2018.9029]

72 **Li WC**, Wu YQ, Gao B, Wang CY, Zhang JJ. MiRNA-574-3p inhibits cell progression by directly targeting CCND2 in colorectal cancer. *Biosci Rep* 2019; **39** [PMID: 31729531 DOI: 10.1042/BSR20190976]

73 **He X**, Chen SY, Yang Z, Zhang J, Wang W, Liu MY, Niu Y, Wei XM, Li HM, Hu WN, Sun GG. miR-4317 suppresses non-small cell lung cancer (NSCLC) by targeting fibroblast growth factor 9 (FGF9) and cyclin D2 (CCND2). *J Exp Clin Cancer Res* 2018; **37**: 230 [PMID: 30227870 DOI: 10.1186/s13046-018-0882-4]

74 **Sun CC**, Zhang L, Li G, Li SJ, Chen ZL, Fu YF, Gong FY, Bai T, Zhang DY, Wu QM, Li DJ. The lncRNA PDIA3P Interacts with miR-185-5p to Modulate Oral Squamous Cell Carcinoma Progression by Targeting Cyclin D2. *Mol Ther Nucleic Acids* 2017; **9**: 100-110 [PMID: 29246288 DOI: 10.1016/j.omtn.2017.08.015]

75 **Wang Y**, Xue J, Kuang H, Zhou X, Liao L, Yin F. microRNA-1297 Inhibits the Growth and Metastasis of Colorectal Cancer by Suppressing Cyclin D2 Expression. *DNA Cell Biol* 2017; **36**: 991-999 [PMID: 28933597 DOI: 10.1089/dna.2017.3829]

76 **Mo X**, Cao Q, Liang H, Liu J, Li H, Liu F. MicroRNA-610 suppresses the proliferation of human glioblastoma cells by repressing CCND2 and AKT3. *Mol Med Rep* 2016; **13**: 1961-1966 [PMID: 26782072 DOI: 10.3892/mmr.2016.4760]

77 **Yu WF**, Wang HM, Lu BC, Zhang GZ, Ma HM, Wu ZY. miR-206 inhibits human laryngeal squamous cell carcinoma cell growth by regulation of cyclinD2. *Eur Rev Med Pharmacol Sci* 2015; **19**: 2697-2702 [PMID: 26221902]

78 **Zhang L**, Liu X, Jin H, Guo X, Xia L, Chen Z, Bai M, Liu J, Shang X, Wu K, Pan Y, Fan D. miR-206 inhibits gastric cancer proliferation in part by repressing cyclinD2. *Cancer Lett* 2013; **332**: 94-101 [PMID: 23348698 DOI: 10.1016/j.canlet.2013.01.023]

79 **Xie T**, Wang Z, Zhao Q, Bai Q, Zhou X, Gu Y, Peng W, Wang H. Machine Learning-Based Analysis of MR Multiparametric Radiomics for the Subtype Classification of Breast Cancer. *Front Oncol* 2019; **9**: 505 [PMID: 31259153 DOI: 10.3389/fonc.2019.00505]

80 **Sharma S**, Mehra R. Conventional Machine Learning and Deep Learning Approach for Multi-Classification of Breast Cancer Histopathology Images-a Comparative Insight. *J Digit Imaging* 2020 [PMID: 31900812 DOI: 10.1007/s10278-019-00307-y]

81 **Cancer Genome Atlas Research Network**, Weinstein JN, Collisson EA, Mills GB, Shaw KR, Ozenberger BA, Ellrott K, Shmulevich I, Sander C, Stuart JM. The Cancer Genome Atlas Pan-Cancer analysis project. *Nat Genet* 2013; **45**: 1113-1120 [PMID: 24071849 DOI: 10.1038/ng.2764]

82 **Cancer Genome Atlas Research Network**. Comprehensive molecular characterization of urothelial bladder carcinoma. *Nature* 2014; **507**: 315-322 [PMID: 24476821 DOI: 10.1038/nature12965]

**Footnotes**

**Conflict-of-interest statement:** The authors have no financial conflicts of interest.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

**Manuscript source:** Invited manuscript

**Peer-review started:** January 25, 2020

**First decision:** April 19, 2020

**Article in press:** May 12, 2020

**Specialty type:** Immunology

**Country/Territory of origin:** Israel

**Peer-review report’s scientific quality classification**

Grade A (Excellent): 0

Grade B (Very good): B

Grade C (Good): C

Grade D (Fair): 0

Grade E (Poor): 0

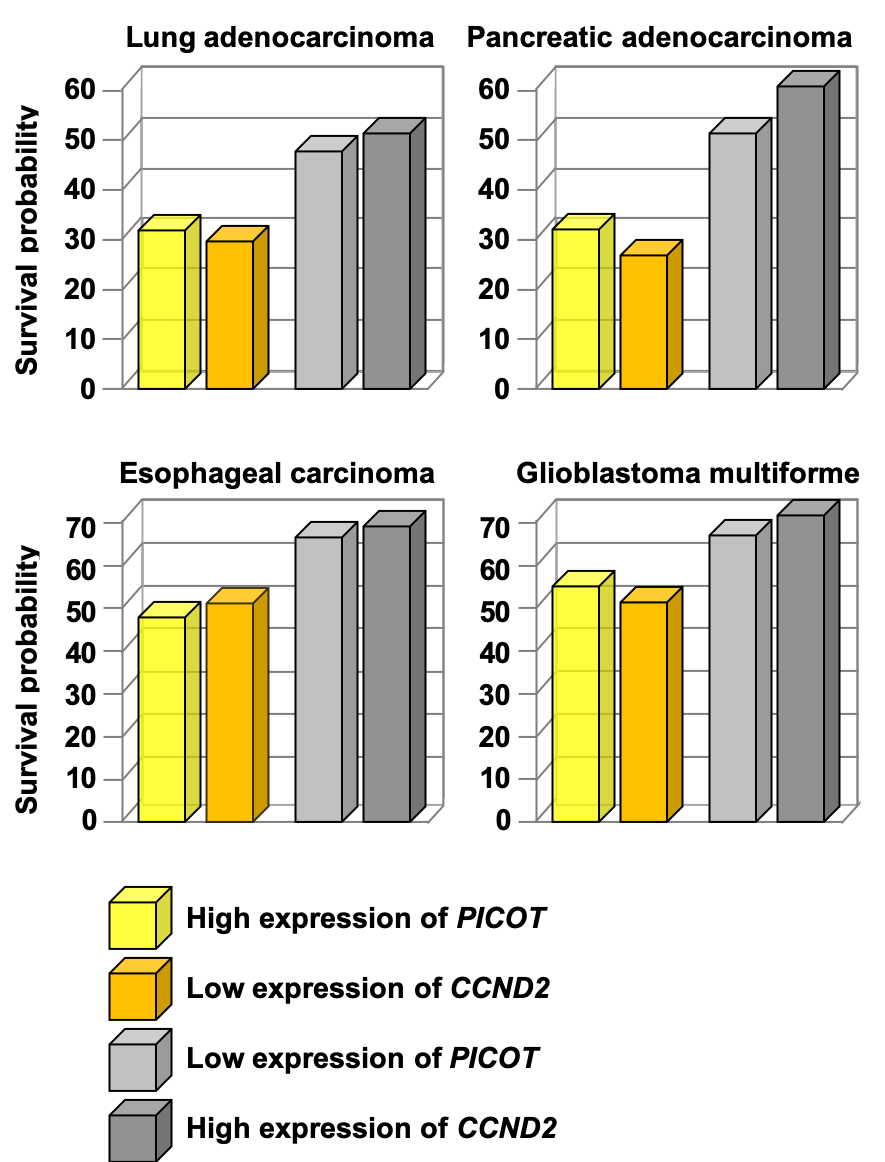
**P-Reviewer:** Pop TL, Velikova TV **S-Editor:** Dou Y **L-Editor:** A **E-Editor:** Xing YX

**Figure Legends**

**手机屏幕的截图

描述已自动生成**

**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; me3: 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.



**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.