Experimental and Molecular Pathology 108 (2019) 73–79

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

Plakophilin 1 is methylated and has a tumor suppressive activity in human lung cancer

Daniela Haase[a,1], Tiantian Cui[a,1,2], Linlin Yang[a,2], Yunxia Ma[a], Hongyu Liu[b], Bernhard Theis[a], Iver Petersen[a,3], Yuan Chen[a,*]

[a] *Section Pathology of the Institute of Forensic Medicine, University Hospital Jena, Friedrich Schiller University Jena, Am Klinikum 1, 07747 Jena, Germany*

[b] *Hematology, Oncology and Tumorimmunology, Charité Campus Benjamin Franklin, Hindenburgdamm 30, 12200 Berlin, Germany*

ARTICLE INFO

Keywords:
Lung cancer
Plakophilin 1
DNA methylation
Tumor suppressor
Tissue microarray

ABSTRACT

Background: Plakophilin 1 (PKP1) is an important plaque component of desmosomes, major intercellular adhesive junctions that act as anchorage points for intermediate filaments. Abnormal expression of PKP1 was observed in various types of cancer, however so far its function in lung cancer has not yet been elucidated.
Methods: The expression of PKP1 was analyzed by RT-PCR and western blotting in lung cancer cell lines. The protein expression of PKP1 was evaluated by immunohistochemistry in tissue microarray. The epigenetic mechanism of PKP1 was explored by demethylation test, bisulfite sequencing and Methylation-Specific-PCR. The function of PKP1 was investigated by stable transfection with an expression vector.
Results: We found that PKP1 was downregulated in 6 out of 8 lung cancer cell lines, and downregulation of PKP1 was associated with DNA hypermethylation. In advanced primary lung tumor samples, higher expression of PKP1 was significantly associated with favorable clinical outcome (p = .003). Ectopic expression of PKP1 inhibited cell proliferation, colony formation, migration/invasion and enhanced apoptosis. These phenomena are accompanied by increased caspase 3/7 activities and cleaved PARP-1 as well as decreased extracellular signal–regulated kinase (ERK) activity.
Conclusion: Taken together, our data suggest that PKP1 is a novel tumor suppressor and its protein expression might be a potential prognostic marker for patients with advanced lung cancer.

1. Introduction

Lung cancer is the most frequent cause of cancer-related death in the USA with more than one-quarter (25.3%) estimated death due to lung cancer in 2018 (Siegel et al., 2018). Lung cancer is divided into two broad histologic classes: small cell lung cancer (SCLC) accounting for 15% of lung cancer cases and non-small cell lung cancer (NSCLC) containing adenocarcinoma, squamous cell carcinoma and large cell carcinoma, which occurs in up to 85% of all lung cancer diagnosed. Although much progress has been made in lung cancer research, screening and therapy in recent years, the five-year survival rates have not been largely increased, varying from 4 to 17% depending on stage and regional differences (Hirsch et al., 2017). A better understanding of molecular mechanisms underlying lung cancer development provides opportunities in advanced therapeutic regime for management of the fatal disease.

Desmosomes confer strong intercellular adhesion through desmosomal cadherins which interact with intracellular linker proteins like plakophilins (PKPs) 1–3 to anchor the intermediate filaments (Rietscher et al., 2016). PKPs are constitutively located in the desmosomal plaque and form a distinct subgroup of the armadillo family (Schmidt and Jäger, 2005). PKPs localize not only in cell-cell junctions but also in the nucleus and the cytoplasm, implying their multiple cellular activities within the cell (Dusek and Attardi, 2011). In human cancer, PKPs play diverse roles. For instance, phosphorylation of PKP1 regulates epidermal differentiation and skin tumorigenesis (Lee et al., 2017). PKP2 is considered a novel Wnt/β-catenin target gene in colon cancer-associated fibroblasts and may modulate Wnt activity in the tumor cells (Niell et al., 2018). Increased PKP3 expression was positively correlated with node positivity and tumor grade in breast cancer (Demirag et al.,

---

[*] Corresponding author at: Institute of Forensic Medicine, Section Pathology, University Hospital Jena, Am Klinikum 1, 07747 Jena, Germany.
E-mail address: Yuan.Chen@med.uni-jena.de (Y. Chen).

[1] These authors contributed equally to the work.

[2] Present address: Department of Radiation Oncology, Arthur G. James Hospital/Ohio State Comprehensive Cancer Center, Columbus, OH, 43210, USA.

[3] Present address: Institute of Pathology, SRH Wald-Klinikum Gera, Str. des Friedens 122, 07548 Gera, Germany.

https://doi.org/10.1016/j.yexmp.2019.04.001
Received 13 March 2019; Received in revised form 28 March 2019; Accepted 1 April 2019

2012). In lung cancer, PKP2 and PKP3 seem to exert the oncogenic function with evidence that PKP2 promotes tumor development by enhancing epidermal growth factor receptor dimerization and activation (Arimoto et al., 2014), and high level of PKP3 expression was associated with poor survival as well as disease stage and node status (Furukawa et al., 2005). However, so far the functional role of PKP1 in lung cancer has not yet been elucidated. In this study, we analyzed the expression of PKP1, explored the epigenetic regulation and investigated the function of PKP1 in human lung cancer.

2. Materials and methods

2.1. Cell lines, cell culture and drug treatment

Human bronchial epithelial cells (HBECs) were purchased from Clonetics (San Diego, CA, USA) and cultured in BEG media (Clonetics). Human lung cancer cell lines including SCLC (COLO668 and COLO677) and NSCLC (H157, H226, H1299, H1650, H2170 and H2228) were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and the German Collection of Microorganisms and Cell Culture (DSMZ, Braunschweig, Germany). The cells were cultured with RPMI 1640 medium containing 10% (*v*/v) fetal bovine serum (FBS) and maintained in a humidified atmosphere with 5% CO_2 at 37 °C.

For demethylation test, cell lines (H157, H226, H2170 and H1299) were cultured on 6-well plates. At 50% confluence, they were treated with 5, 10 or 20 μM of 5-aza-2′-deoxycytidine (Sigma Chemical Co., St Louis, USA) on days 0, 2 and 4. Cells were then harvested for total RNA isolation. For apoptosis assay, cells were treated with apoptotic inducer gemcitabine (0.5 μM, Merck Darmstadt, Germany) for 48 h.

2.2. RNA extraction, RT-PCR and quantitative RT-PCR

RNA was extracted from cell lines using the peqGOLD TriFast™ (VWR, Darmstadt Germany) following the manufacturer's instruction. Total RNA (500 ng) was applied for reverse transcription using QuantiTect® Reverse Transcription Kit (Qiagen, Hilden, Germany).

PCR amplification of PKP1 was carried out under the following conditions: 94 °C 1 min, 53 °C 30 s, 72 °C 30 s, for 30 cycles, with initial denaturation at 95 °C 15 min and final elongation at 72 °C 7 min.

Quantitative RT-PCR was conducted on the Rotor-Gene 6000 (Qiagen) using FastStart Universal SYBR Green Master (Roche, Mannheim, Germany) according to the manufacturer's guide.

The Housekeeping gene Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as control. Primer sequences are shown in Supplementary Table 1.

2.3. Bisulfite treatment, bisulfite sequencing and methylation-specific-PCR

Isolation of genomic DNA and bisulfite treatment were performed as previously described (Chen et al., 2015).

To determine the methylation pattern of PKP1, bisulfite sequencing (BS) was carried out. One pair of primer (Supplementary Table 1) was used to amplify bisulfite modified both methylated and unmethylated genomic DNA in the promoter region (from −378 to −260 bp and from −182 to -7 bp; ENSG00000081277) containing 37 CpG dinucleotides. PCR was carried out with the following conditions: 95 °C 15 min, 40 cycles of 95 °C 30 s, 58 °C 30 s, and 72 °C 45 s, with a final extension at 72 °C for 7 min. The PCR products were purified using a DNA clean & concentration kit (Zymo Research, Freiburg, Germany) and sequenced by capillary electrophoresis (LGC, Berlin, Germany).

For methylation-specific-PCR (MSP) analysis, two pairs of primers (Supplementary Table 1) were designed for amplification of methylated or unmethylated allele, respectively. PCR condition is similar to that used for BS.

2.4. Western blot

Western blot was performed as described previously (Chen et al., 2015). Thirty microgram proteins were electrophoresed on 10% SDS-PAGE gels and blotted on nitrocellulose membrane. Antibodies applied in the study were listed in Supplementary Table 2. Signals were visualized using an enhanced chemiluminescence detection system (GE healthcare, Munich, Germany).

2.5. Tissue microarray (TMA) construction and immunohistochemistry

A total of 208 primary lung tumor specimens obtained from the University Hospital Jena and University Hospital Charité from 2000 to 2010 were included for the construction of TMA. None of the patients underwent adjuvant radiotherapy or chemotherapy before surgery. The study has been approved by the local ethical committee of University Hospital Jena (Nr: 3815-07/13).

TMA was constructed using a manual tissue arrayer (Beecher Instruments, Woodland, USA) and immunohistochemistry (IHC) was carried out to analyze the protein expression of PKP1 on the TMA as previously described (Chen et al., 2015). IHC was scored semi-quantitatively as negative (0% positively stained cells, score 0), weak (< 10% positively stained cells; score 1), moderate 10–25% positively stained cells; score 2), or strong (> 25% positively stained cells; score 3). For statistical evaluation, scores 0 and 1 together were considered negative, while scores 2 and 3 together were thought as positive.

2.6. Stable transfection

An expression vector containing the full-length human-PKP1 cDNA purchased from Origene (Rockville, Maryland, USA) was stably transfected into H157 and H2170 following the instruction of X-tremeGENE HP DNA Transfection Reagent Protocol (Sigma Aldrich, Munich, Germany).

2.7. Cell proliferation, colony formation, apoptosis, cell migration and invasion assays

To compare the cell proliferative rate between PKP1 transfectants and mock transfectant (empty vector), cells (2×10^4/well) were seeded into 96-well plates and counted after 24 h and on days 2, 3, 4, 5, 6, 7 using the Cell Proliferation ELISA BrdU Kit (Roche, Mannheim, Germany) and an automated luminescence microplate reader (LumiStar Galaxy, BMG Labtech, Germany).

For colony formation assay, cells (500 cells/well) were seeded into 6-well plates. After 14 days, colonies were stained with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and counted.

Apoptosis assay was carried out by using an APOPercentage™ Apoptosis Assay Kit (Biocolor Ltd., UK) according to the manufacturer's guide. Concentration of intracellular dye was measured using a microplate colorimeter (TECAN sunrise, Tecan, Switzerland) at the absorbance of 540 nm.

To confirm the results from apoptosis assay, the activity of caspase 3/7 was analyzed by using a Caspase-Glo® 3/7 Assay Kit (Promega, Mannheim, Germany).

Migration and invasion assay was performed as previously described (Chen et al., 2015). For migration assay, cells (5×10^4/well) were re-suspended in 500 μl of RPMI1640 medium and placed in the upper transwell chamber (8 μm pore size, BD Biosciences, Franklin Lakes, NJ, USA). After incubation at 37 °C for 24 h, non-migratory cells were removed and migratory cells were fixed with methanol (−20 °C) for 10 min and stained with 0.5% crystal violet for 3 min at room temperature for calculation. For invasion assay, cells (5×10^4/well) were cultured in FBS free medium for 24 h and seeded in matrigel-coated transwell chambers (BD Biosciences, Heidelberg, Germany). One

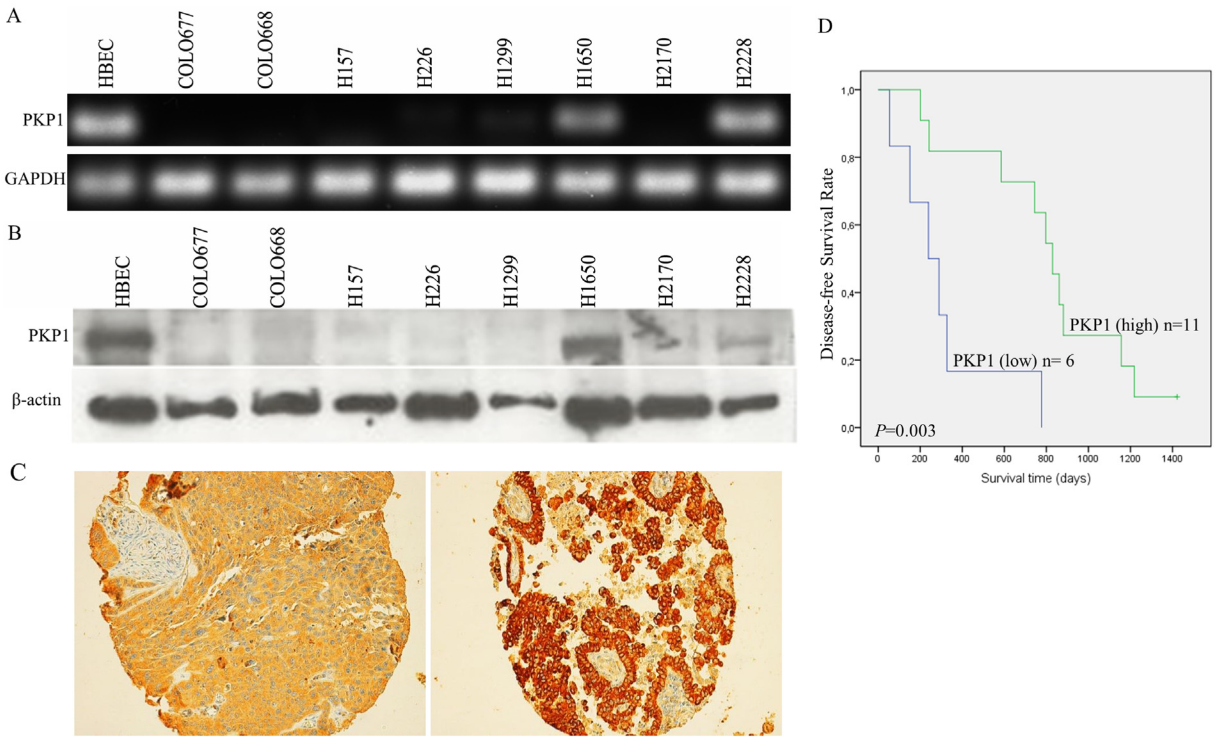

Fig. 1. Expression analysis of PKP1 in lung cancer. (A) PKP1 mRNA expression in 8 lung cancer cell lines was analyzed by RT-PCR. GAPDH was used as control. (B) PKP1 protein expression was analyzed by western blotting in lung cancer cell lines. β-actin was used as loading control. (C) Representative images of PKP1 protein expression in primary lung squamous cell carcinoma (left) and adenocarcinoma (right). (D) Kaplan–Meier curves showed that high expression of PKP1 was significantly related to improved disease-free survival (p = .003) in patients with advanced stage of lung cancer.

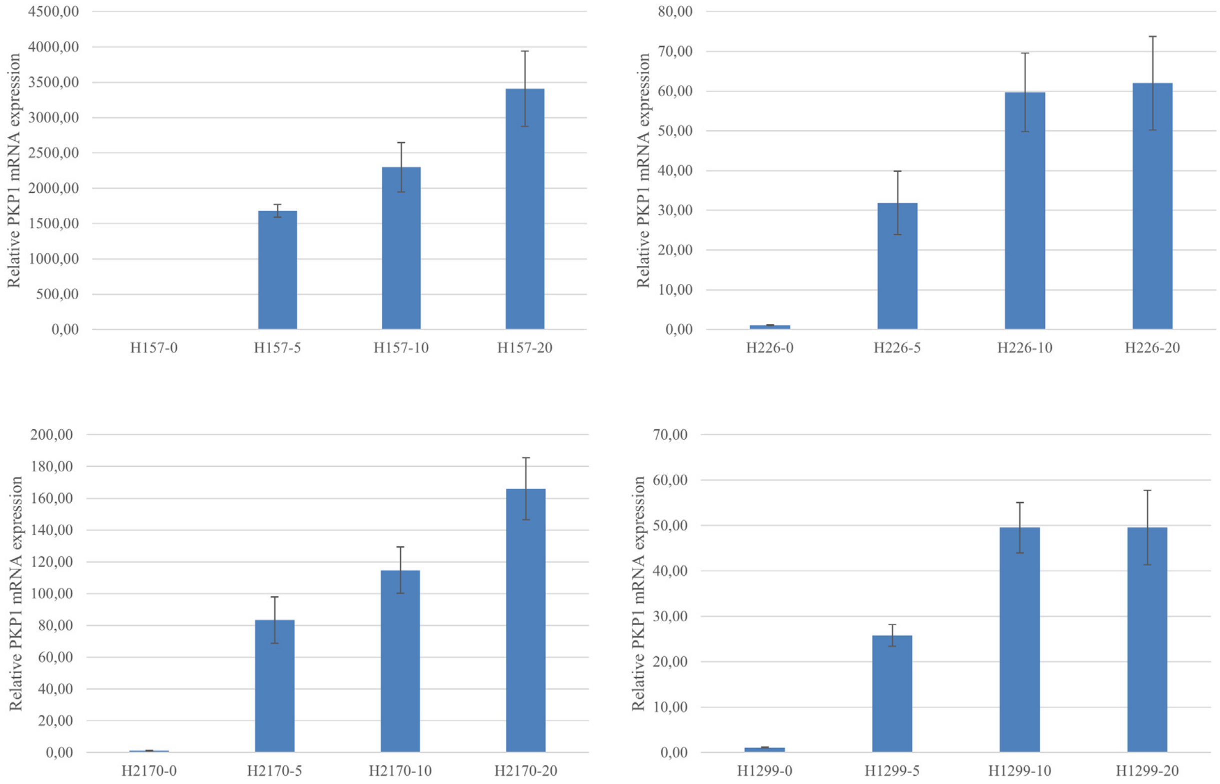

Fig. 2. Restoration of PKP1 mRNA expression after treatment with 5-aza-2′-deoxycytidine. Quantitative RT-PCR showed that after treated with 5-aza-2′-deoxycytidine, a DNA methyltransferase inhibitor, in the 4 lung cancer cell lines (H157, H226, H2170 and H1299) the mRNA expression was upregulated in a dose-dependent manner. The concentrations of 5-aza-2′-deoxycytidine are indicated.

Table 1
Methylation patterns of PKP1 DNA in promoter region and exon 1 in lung cancer cell lines.

CpG position / cell line	-126	-114	-107	-89	-69	-60	-37	-35	-18	-16	-14	-9	69	85	123	125	132	145	155	164	175	185	189	197	204	209	232	236	238	240	247	254
HBEC	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
COCO668	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	●	●	●	●	●	●	•	•	•	•	•	•	•	•
COLO677	•	•	●	●	●	●	•	•	●	●	●	●	•	•	•	●	•	●	●	●	•	•	•	•	•	•	•	●	●	●	•	•
H157	•	•	•	•	•	•	●	●	●	●	●	●	•	•	•	●	•	•	•	•	•	•	●	•	•	•	•	●	●	•	●	•
H226	●	•	•	•	●	●	●	●	●	●	●	●	•	•	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
H1299	•	•	•	●	●	●	●	●	●	●	●	●	•	•	•	•	•	●	●	●	•	•	•	●	•	•	•	●	●	●	•	•
H1650	○	○	○	○	•	•	•	•	•	•	•	•	○	○	○	○	○	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
H2170	•	•	•	•	•	•	•	•	•	•	•	•	○	○	○	○	○	●	•	•	●	●	●	●	●	●	●	●	●	●	●	●
H2228	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	•	•	○	○	○	○	○	○	○	○	○

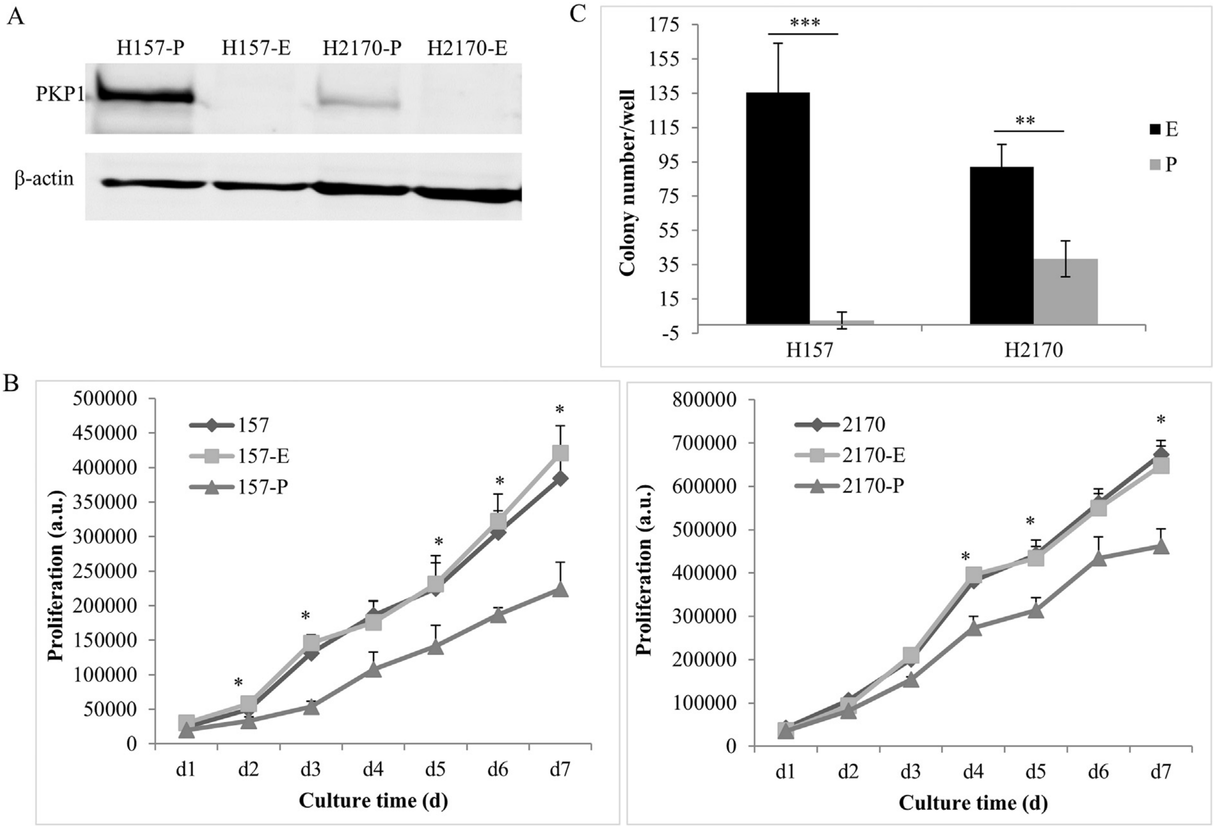

Fig. 3. Effects of ectopic PKP1 expression on lung cancer cell proliferation and colony formation. (A) Ectopic PKP1 expression in the stably transfected cell lines H157 and H2170 was confirmed by western blot analysis. (B) Proliferation assay showed that PKP1 positive transfected cells inhibited cell proliferation compared to mock control cells and parent cells. (C) Colony formation assay showed that PKP1 overexpression resulted in decreased colony formation compared to mock control. The data presented are the means ± SE from three independent experiments. $^{**}p < .01$ and $^{***}p < .001$ when analyzed with Student's *t*-test.

day later, uninvaded cells were removed and the invaded cells were stained for calculation. All experiments were conducted in triplicate.

2.8. Statistical analysis

Statistical analysis was performed using the statistical software package SPSS21 (SPSS, Chicago, USA). Two-tailed chi-square ($\chi2$) test or Fisher's exact test was performed to analyze the correlation between PKP1 protein expression/methylation and clinicopathological parameters. Student's *t*-test was carried out to detect the differences between PKP1 transfectants and mock control. *P* values < .05 were considered statistically significant.

3. Results

3.1. Analysis of PKP1 expression in lung cancer cell lines and primary lung tumors

PKP1 mRNA expression was downregulated in the majority of lung cancer cell lines compared with HBEC (Fig. 1A). Protein expression of PKP1 was consistent with the mRNA levels (Fig. 1B).

In 208 cases with primary lung tumors, less than half (44.7%) of cases exhibited positive staining of PKP1 protein. Representative images of PKP1 protein expression are present in Fig. 1C with positive staining in both cytoplasm and nucleus. The PKP1 protein expression was not significantly associated with clinicopathological parameters including tumor grading, size, lymph node metastasis and sub-classification. However, Kaplan–Meier survival curves revealed that higher PKP1 expression was significantly related to longer survival time in patients with advanced stage tumors ($p = .003$; Fig. 1D).

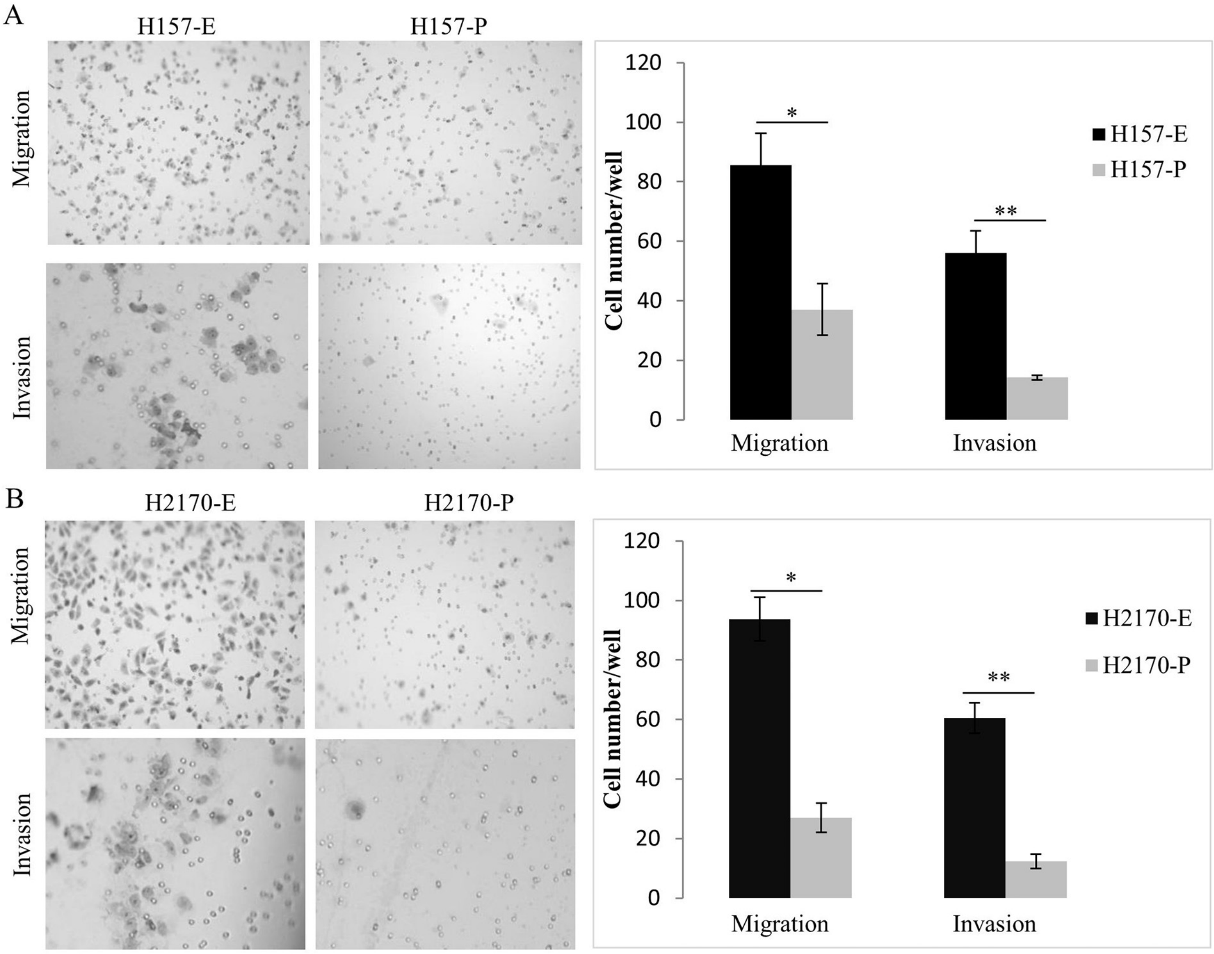

Fig. 4. Effects of ectopic PKP1 expression on lung cancer cell migration and invasion. (A) Migration and invasion assay showed that overexpression of PKP1 reduced the migratory and invasion potential of H157 (50 × objective, left); quantification of migrated and invaded cells of H157 (right). (B) Migration and invasion assay showed that overexpression of PKP1 reduced the migratory and invasive potential of H2170 (50 × objective, left); quantification of invaded cells (right).

3.2. Analysis of PKP1 DNA methylation in lung cancer

To explore whether PKP1 downregulation could be caused by DNA methylation, we treated the 4 NSCLC cell lines H157, H226, H1299 and H2170 which showed very low expression of PKP1 with 5-aza-2′-deoxycytidine, a DNA methyltransferase inhibitor. It turned out that PKP1 mRNA expression was markedly restored in all these cell lines in a dose dependent manner (Fig. 2), indicating that PKP1 itself or its upstream regulatory genes might be methylated. To further analyze the methylation status of PKP1 DNA, we performed bisulfite sequencing. A heterogeneous methylation pattern of PKP1 turned out in the promoter region (from −126 to −9 bp) and exon 1 (from +69 to +254) including 32 CpG dinucleotides. As expected, in normal cells HBEC, the PKP1 DNA was totally unmethylated. In H2228 with endogenous expression of PKP1, the PKP1 DNA was unmethylated in the promoter region and in the majority of the CpG sites of exon 1. In H1650 with PKP1 expression, PKP1 DNA is either unmethylated or only partially methylated. On the contrary, PKP1 DNA was fully or partially methylated in the most of the CpG sites in the PKP1 downregulated cell lines (Table 1).

The results from BS were confirmed by methylation-specific-PCR (MSP) (data not shown) indicating the utility of the primer. In the analysis of the methylation status of PKP1 in primary lung tumor, we found that 41 out of 46 samples (89.1%) exhibited full or partial methylation of PKP1 DNA, while 5 samples (10.9%) were unmethylated. No significant association between PKP1 DNA methylation and clinicopathological parameters was found (Supplementary Table 3).

3.3. PKP1 inhibits tumor cell proliferation, colony formation, migration and invasion

To investigate the functional role of PKP1 in lung cancer cells, an expression vector containing the full-length cDNA of PKP1 was stably transfected into H157 and H2170 lacking endogenous expression of PKP1. After stable transfection, positive transfectants H157-P and H2170-P were selected by western blotting (Fig. 3A).

Proliferation assay revealed that PKP1 overexpression markedly inhibited tumor cell proliferation compared to mock control cells H157-E and H2170-E (Fig. 3B). Colony formation assay showed that the number of colony was significantly reduced in PKP1 transfectants compared to control (Fig. 3C).

Migration and invasion assay revealed that the number of the cells migrated and invaded into the lower chamber was significantly reduced in PKP1 transfected cells compared to mock cells (Fig. 4).

We further analyzed the MAPK pathway by western blot analysis. It turned out that the classical MAP kinase, extracellular signal–regulated kinase (ERK), was inhibited upon PKP1 overexpression in H157 (Fig. 5A).

3.4. PKP1 enhances apoptosis

To investigate the influence of PKP1 overexpression on apoptosis, we performed apoptosis assay. As shown in Fig. 5B, PKP1 overexpression resulted in more apoptosis in H157 compared to mock cells, which was accompanied by increased caspase 3/7 activity (Fig. 5C).

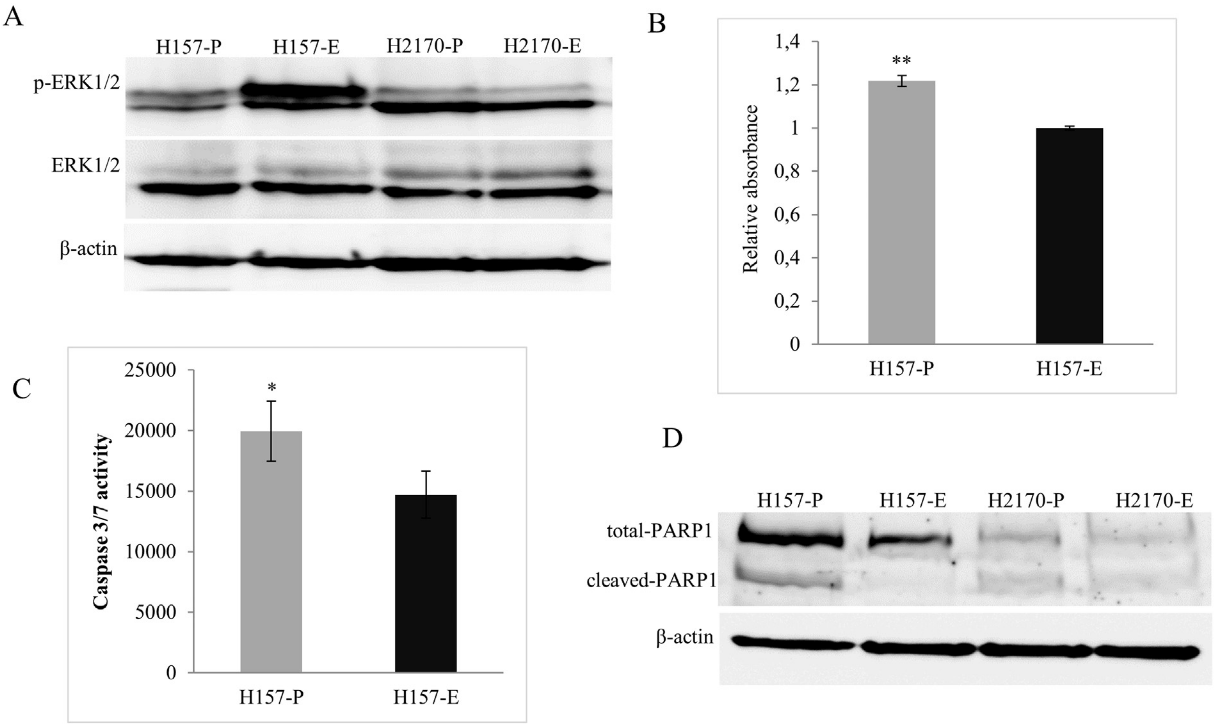

Fig. 5. Effects of ectopic PKP1 expression on apoptosis in lung cancer cells. (A) Western blot analysis revealed that ectopic PKP1 expression led to decreased phosphorylated level of ERK1/2 (p-ERK1/2) in H157. β-actin was used as loading control. (B) Apoptosis assay showed a significantly higher absorbance in PKP1 transfected H157 cells compared to mock cells. (C) Caspase 3/7 activity assay showed an enhanced caspase 3/7 activity in the PKP1 transfected H157 cell line compared to mock cells. (D) Western blot analysis revealed that the cleaved PARP1 level was increased in H157 transfectant compared to mock cells, while no alteration of cleaved PARP1 was detected in H2170 transfectant compared to mock cells.

However, in H2170, this effect was not observed (data not shown).

We further analyzed the expression of one of the apoptotic markers, the cleaved PARP-1 by western blotting. Consistent with the results from apoptosis assay, cleaved PARP-1 level was only enhanced in H157 transfectants, but not in H2170 transfectants (Fig. 5D).

4. Discussion

Plakophilin 1, a member of the armadillo multigene family, contains two functionally distinct domains: the head domain, playing a role in organizing the desmosomal plaque in cells, and the armadillo repeat domain, involved in regulating the dynamics of the actin cytoskeleton (Hatzfeld et al., 2000). Besides, PKP1 participates in RNA metabolism, protein translation and cell signaling (Hatzfeld et al., 2014). Mutations in PKP1 have been associated with the ectodermal dysplasia/skin fragility syndrome (Olivry et al., 2012). In this study, we focused on the functional role of PKP1 in human lung cancer.

Expression analysis showed that PKP1 was downregulated in the majority of lung cancer cell lines on both mRNA and protein levels. In primary lung tumors, the high expression of PKP1 protein was significantly associated with favorable clinical outcome in patients with advanced stage of disease, indicating a prognostic value of PKP1. However, the sample size included for the analysis is small. In future studies, it is necessary to increase sample size to clarify the clinical significance of PKP1 in lung cancer.

Cancer initiation and development are closely associated with profound changes in DNA methylation (Sharma et al., 2010). DNA methylation is one of the mechanisms responsible for gene silencing. In our study, the results from bisulfite sequencing and methylation-specific-PCR clearly point out that PKP1 downregulation is related to PKP1 DNA methylation in lung cancer. Aberrantly methylated PKP1 was thought to contribute to the progression of Barrett's esophagus to esophageal adenocarcinoma (Kaz et al., 2012), and in NSCLC, methylation of PKP1 was identified by combination of in silico and experimental approaches (Kusakabe et al., 2010). In primary lung tumors, however, PKP1 negative staining is not significantly correlated to PKP1 DNA methylation, indicating that besides DNA methylation, other mechanisms might play a role in the gene silencing of PKP1 in lung cancer.

Like other desmosomal proteins, the role of PKPs in cancer is not well understood. While the expression of PKPs1–3 was increased in certain cancers of various origin, the loss/reduction of PKPs1–3 was observed in the development and/or progression of the same or other cancers, indicating that their contribution to cancer might be context-dependent and determined by the status of distinct signaling pathways that affect PKP functions (Dusek and Attardi, 2011; Hatzfeld et al., 2014). In line with the tumor inhibitory effect of PKP1 on skin cancer and esophageal adenocarcinoma (Lee et al., 2017; Kaz et al., 2012), ectopic expression of PKP1 by stable transfection resulted in reduced cell proliferation, migration/invasion and enhanced apoptosis, suggesting its tumor suppressive activity in lung cancer. It is not surprising to see this, since loss of desmosomal protein would affect intercellular cohesion, facilitates migration and proliferation, and thus promotes tumor growth and/or metastasis. By using the same cell model, the tumor suppressive function of other desmosomal proteins including desmocollin 3 and desmoplakin was also characterized in our previous studies (Cui et al., 2012; Yang et al., 2012).

It has been reported that insulin/IGF-1-signaling via the PI3K/AKT pathway induced the phosphorylation of PKP1, leading to the stabilization of the cytoplasmic pool which in turn increase in cellular proliferation (Wolf et al., 2013). Recently, PKP1 has been considered to be a novel Wnt/β-catenin signaling regulator for ameloblast differentiation (Miyazaki et al., 2016). In our cell model, no alterations in PI3K/AKT or Wnt/β-catenin signaling were detected after overexpression of PKP1 by transfection (data not shown). Instead, we found that the activity of ERK1/2, a downstream molecule of mitogen-activated protein kinase (MAPK) signaling, was inhibited upon ectopic expression of

PKP1 in H157 transfectant, in which an increased apoptotic rate and cleaved PARP1 expression was observed. Since MAPK pathway links extracellular signals to the machinery that controls fundamental cellular processes including proliferation, differentiation, migration and apoptosis (Dhillon et al., 2007), we speculate that PKP1 exerts its tumor suppressive function probably through inhibition of MAPK pathway in lung cancer cells.

In summary, this is the first study reporting the tumor suppressive activity of PKP1 in lung cancer. Tumor suppressors are going to be targets for therapeutics (Wang et al., 2006; Morris and Chan, 2015). We hope that the function of PKP1 may stimulate further investigations of desmosomal proteins in carcinogenesis and development of therapeutic strategies based on cell-cell adhesion in human cancer.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yexmp.2019.04.001.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgements

The work was supported by a junior project grant from Interdisciplinary Centre for Clinical Research in Jena University Hospital, Germany.

References

Arimoto, K., et al., 2014. Plakophilin-2 promotes tumor development by enhancing ligand-dependent and -independent epidermal growth factor receptor dimerization and activation. Mol. Cell. Biol. 34, 3843–3854.

Chen, Y., et al., 2015. HOPX is methylated and exerts tumour-suppressive function through Ras-induced senescence in human lung cancer. J. Pathol. 235, 397–407.

Cui, T., et al., 2012. The p53 target gene desmocollin 3 acts as a novel tumor suppressor through inhibiting EGFR/ERK pathway in human lung cancer. Carcinogenesis 33, 2326–2333.

Demirag, G.G., et al., 2012. Expression of Plakophilins (PKP1, PKP2, and PKP3) in breast cancers. Med. Oncol. 29, 1518–1522.

Dhillon, A.S., et al., 2007. MAP kinase signalling pathways in cancer. Oncogene 26, 3279–3290.

Dusek, R.L., Attardi, L.D., 2011. Desmosomes: new perpetrators in tumour suppression. Nat. Rev. Cancer 11, 317–323.

Furukawa, C., et al., 2005. Plakophilin 3 oncogene as prognostic marker and therapeutic target for lung cancer. Cancer Res. 65, 7102–7110.

Hatzfeld, M., et al., 2000. The function of plakophilin 1 in desmosome assembly and actin filament organization. J. Cell Biol. 149, 209–222.

Hatzfeld, M., et al., 2014. Plakophilins in desmosomal adhesion and signaling. Cell Commun. Adhes. 21, 25–42.

Hirsch, F.R., et al., 2017. Lung cancer: current therapies and new targeted treatments. Lancet 389, 299–311.

Kaz, A.M., et al., 2012. Aberrantly methylated PKP1 in the progression of Barrett's esophagus to esophageal adenocarcinoma. Genes Chromosom. Cancer 51, 384–393.

Kusakabe, M., et al., 2010. Identification of G0S2 as a gene frequently methylated in squamous lung cancer by combination of in silico and experimental approaches. Int. J. Cancer 126, 1895–1902.

Lee, P., et al., 2017. Phosphorylation of Pkp1 by RIPK4 regulates epidermal differentiation and skin tumorigenesis. EMBO J. 36, 1963–1980.

Miyazaki, K., et al., 2016. Plakophilin-1, a novel Wnt signaling regulator, is critical for tooth development and ameloblast differentiation. PLoS One 11, e0152206.

Morris, L.G., Chan, T.A., 2015. Therapeutic targeting of tumor suppressor genes. Cancer 121, 1357–1368.

Niell, N., et al., 2018. The human PKP2/plakophilin-2 gene is induced by Wnt/β-catenin in normal and colon cancer-associated fibroblasts. Int. J. Cancer 142, 792–804.

Olivry, T., et al., 2012. Deficient plakophilin-1 expression due to a mutation in PKP1causes ectodermal dysplasia-skin fragility syndrome in Chesapeake Bay retriever dogs. PLoS One 7, e32072.

Rietscher, K., et al., 2016. Growth retardation, loss of desmosomal adhesion, and impaired tight junction function identify a unique role of plakophilin 1 in vivo. J. Invest. Dermatol. 136, 1471–1478.

Schmidt, A., Jäger, S., 2005. Plakophilins—hard work in the desmosome, recreation in the nucleus? Eur. J. Cell Biol. 84, 189–204.

Sharma, S., et al., 2010. Epigenetics in cancer. Carcinogenesis 31, 27–36.

Siegel, R.L., et al., 2018. Cancer statistics, 2018. CA Cancer J. Clin. 68, 7–30.

Wang, H., et al., 2006. Targeting loss-of-function mutations in tumor-suppressor genes as a strategy for development of cancer therapeutic agents. Semin. Oncol. 33, 513–520.

Wolf, A., et al., 2013. Insulin signaling via Akt2 switches plakophilin 1 function from stabilizing cell adhesion to promoting cell proliferation. J. Cell Sci. 126, 1832–1844.

Yang, L., et al., 2012. Desmoplakin acts as a tumor suppressor by inhibition of the Wnt/β-catenin signaling pathway in human lung cancer. Carcinogenesis 33, 1863–1870.