



Short Report

Expression of PTEN and pAKT in in Non-Small Cell Lung Cancer

Carina Antfang ¹⁾, Agnes Csanadi ¹⁾, Gian Kayser ¹⁾

¹⁾ Institute of Surgical Pathology, University Medical Center Freiburg, Faculty of Medicine, Freiburg, Germany.

Corresponding author

Gian Kayser

Institute of Surgical Pathology

University Medical Center Freiburg

Breisacher Strasse 115a

D-79106 Freiburg

Email: gian.kayser@uniklinik-freiburg.de

Abstract

Background: Alterations in the PTEN-AKT pathway play important roles indifferent solid carcinomas, e. g. of the breast, prostate and GI-tract. Since contradictory results have been reported for lung cancer mostly studying only one of the two proteins, we aim to elucidate the biological effects of alterations in this pathway in a large and well characterized cohort of non-small cell lung cancer.

Material and Methods: We established an immunohistochemical double stain for PTEN and p-AKT in non-small cell lung carcinomas (NSCLC). This was applied to tissue-microarrays (TMA) including 44 patients.

Results: Our results indicate, that not only alterations in one marker are of biological significance but rather the combination of PTEN-loss and p-AKT overexpression, as well as the intracellular localization of the protein staining. Patients with low-grade histological differentiation, negative lymphonodal status and PTEN-expression showed a significant better overall survival.

Conclusion: We conclude, that PTEN-(over-)expression leads to p-AKT downregulation with better overall survival in nodal negative NSCLC patients.

Keywords: [PTEN-AKT pathway](#); [non-small cell lung carcinoma](#); [immunohistochemical double stain](#); [survival](#).

Virtual Slides:





Introduction

For decades researchers have been trying to understand molecular signaling pathways which may lead to cell growth and tumorigenesis. One of these pathways with which cells control their proliferation and growth includes the oncogene AKT (also named proteinkinase B) and the tumor suppressor gene PTEN (Phosphatase and tensin homolog). AKT and PTEN have long been studied and their effects on biochemical pathways investigated. The aim of this study was to better understand the interaction between these two proteins and their influence in the tumorigenesis of non-small cell lung cancer (NSCLC).

Cells depend on growth signals perceived by its receptors on their outer cell membrane. This signal then gets transduced into the cell via one of its many receptor tyrosine kinases (TKI) e.g. EGFR (epidermal growth factor receptor) or HER2 (human epidermal growth factor receptor 2; Fig. 1). The TKI subsequently usually activates two different pathways. One promotes the cells' proliferation via RAS and RAF which leads to an activation of the MAP kinase signaling pathway. The other activates the PI3K (phosphatidylinositide 3-kinase) which ensures the cell's survival by phosphorylating AKT and setting off the mTOR signaling pathway (1-3).

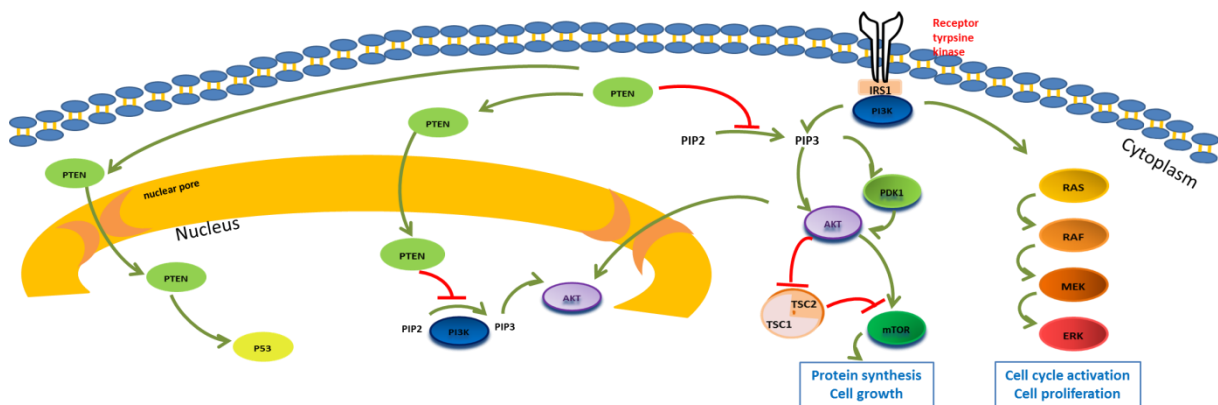


Fig. 1: PI3K Signaling pathway

1.1. The PI3K signaling pathway

The activated PI3K phosphorylates PIP2 (phosphatidylinositol 4,5-bisphosphate) forming PIP3 (Phosphatidylinositol-3,4,5-triphosphate). PIP3 binds AKT which then is able to get



phosphorylated. Various studies suggest that AKT needs to be phosphorylated in a variety of sites in order to be fully activated. A primary activation might be reached by the phosphorylation of AKT at serine 124 and threonine 450, while full activation requires additional phosphorylation at serine 473 and threonine 308 (4). Activated AKT then simultaneously initiates the mTOR (mammalian target of rapamycin) pathway that leads to cell proliferation and inhibits apoptosis by influencing other proteins' functions e.g. activation of NF- κ B and inhibition of BAD (BCL2 Associated Agonist Of Cell Death) (5).

In order for a physiological regulation and stabilization of proliferation, tumor suppressors like PTEN can interfere with these signaling pathways. PTEN breaks PIP3 down into its two components of PIP2 and phosphate and thereby inhibits AKT from being activated. This dephosphorylation plays an important role in inhibiting the cell from undergoing unhindered growth and can even support apoptosis. A loss of PTEN can therefore result in an unstoppable signal transduction via AKT and uncontrollable cell proliferation (3).

PTEN has already been discovered in the 1990's, firstly investigated under the name of MMAC1 (mutated in multiple advanced and epithelial-cell-enriched phosphatase 1) and TEP1 (TGF- β regulated and epithelial-cell-enriched phosphatase 1). It is located on chromosome 10q23 on which deletions in the genome often lead to tumor diseases like breast and prostate cancer (6-8). In various tumor entities, e.g. gliomas, breast cancer and kidney tumors, a loss of PTEN could be observed (3).

PTEN not only influences the PI3K signaling pathway, but also interacts with the tumor suppressor p53. p53 inhibits the cell cycle, strengthens apoptotic signals and is capable of increasing PTEN expression. At the same time PTEN influences p53 by elongating its half-life and stabilizing its DNA binding. Even though it is relatively common to have mutations in either one of these two proteins during tumorigenesis, they rarely exist simultaneously. Presumably, their strong interaction renders it unnecessary to have mutations in both of them (9, 10).



1.2. The sub-cellular localization is important

Furthermore, the location of the proteins within the cell appears to play an important role. PTEN in the cytoplasm mainly inhibits the PI3K signaling pathway using its characteristics as a phosphatase while the same protein in the cell's nucleus seems to coordinate the cell cycle arrest independently of its phosphorylation abilities (11).

AKT mostly gets activated in the cytoplasm and then migrates into the nucleus. Within the nucleus it hinders the cell from undergoing apoptosis and therefore ensures the cell's survival (12). Studies on cardiomyocytes were able to show a positive correlation between AKT activation and estrogens (13). Especially in breast cancer AKT activations could be detected in many cases. Drugs used in this type of cancer like Paclitaxel and Doxorubicin can influence the expression of AKT. This results in a change of sensitivity for other drugs and can even create resistances (14).

Another study was able to show the interaction between pAKT (Thr308) in the nucleus and PTEN in cell cultures. Atorvastatin could induce a rapid migration of PTEN into the nucleus. Simultaneously, pAKT levels dropped significantly. Using immunofluorescence and immunoprecipitation, it could be shown that PTEN migrates to the exact spots in the nucleus where pAKT is found. Here, it binds pAKT and forms a complex (15).

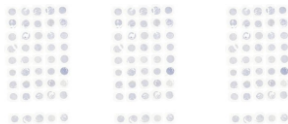
Materials and Methods

In our study we investigated the expression levels of PTEN and pAKT (Thr450) in NSCLC using immunohistochemistry. The stained tissues were microscopically analyzed and rated according to the established H-Score. A special emphasis was placed on the sub cellular localization of the proteins. We then correlated the expression levels to the grading of the tumor, the histological subtype, the overall survival rate and additional patient characteristics. Included in the study were 444 patients, 332 men and 112 women, with a histologically determined NSCLC. Ages ranged from 31 up to 83 years.



Results

The quality of the investigated TMAs is exemplarily shown in the acquired virtual slides



The intra-cellular – localization and intensity of the applied markers can faultlessly be investigated.

PTEN expression was mainly found in the cytoplasm while pAKT was predominantly found in the nucleus. Both proteins were significantly higher expressed in tumor cells in comparison to non-neoplastic lung tissue ($p > 0.001$). Correlating the protein expression with the grading of the tumor especially cytoplasmic PTEN is found to show first an upregulation towards poorer differentiated tumors, but then decreases significantly in un-differentiated large cell lung cancer samples ($p = 0.003$; Fig. 2). pAKT expression was found to be more pronounced with dedifferentiation ($p = 0.009$; Fig. 3). Especially pAKT in the cytoplasm showed a steady increase towards un-differentiation and was significantly higher expressed in high grade tumors ($p = 0.018$).

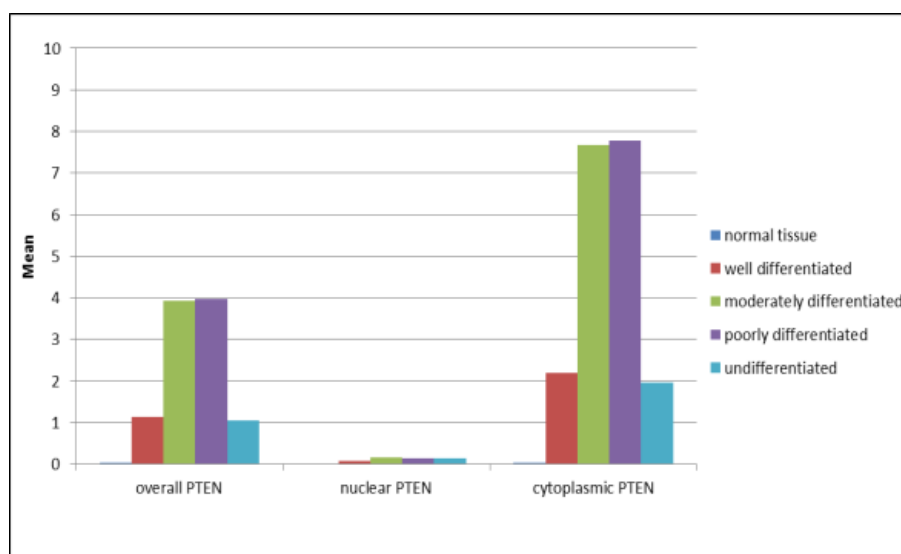


Fig. 2: PTEN expression according to the grading: Cytoplasmic PTEN expression shows upregulation towards poorer differentiated tumours and loss of expression in undifferentiated tumours ($p = 0.003$).

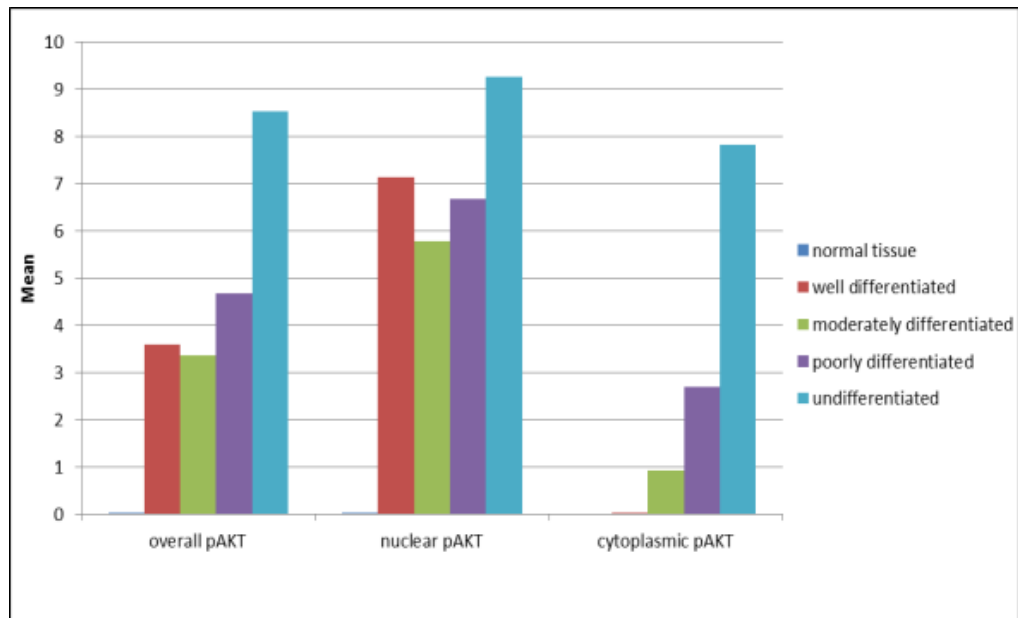


Fig.3: pAKT expression according to the grading: Cytoplasmic pAKT increases with the degree of dedifferentiation ($p=0.009$) while the pAKT expression in the nucleus doesn't show a difference.

Analyzing the correlation between the protein expression and the histological subtype PTEN is significantly higher expressed in adenocarcinoma than in any other histological subtype ($p=0.001$; Fig. 4). Interestingly, pAKT expression was more intense not only in adenocarcinoma, but also in large cell neuroendocrine cancer (LCNEC) compared to other NSCLC subtypes ($p=0.001$). Moreover a co-expression of PTEN and pAKT - only in the combination of cytoplasmic PTEN with nuclear pAKT - in the same cell occurred prevalently in adenocarcinoma ($p=0.001$; Fig. 5).

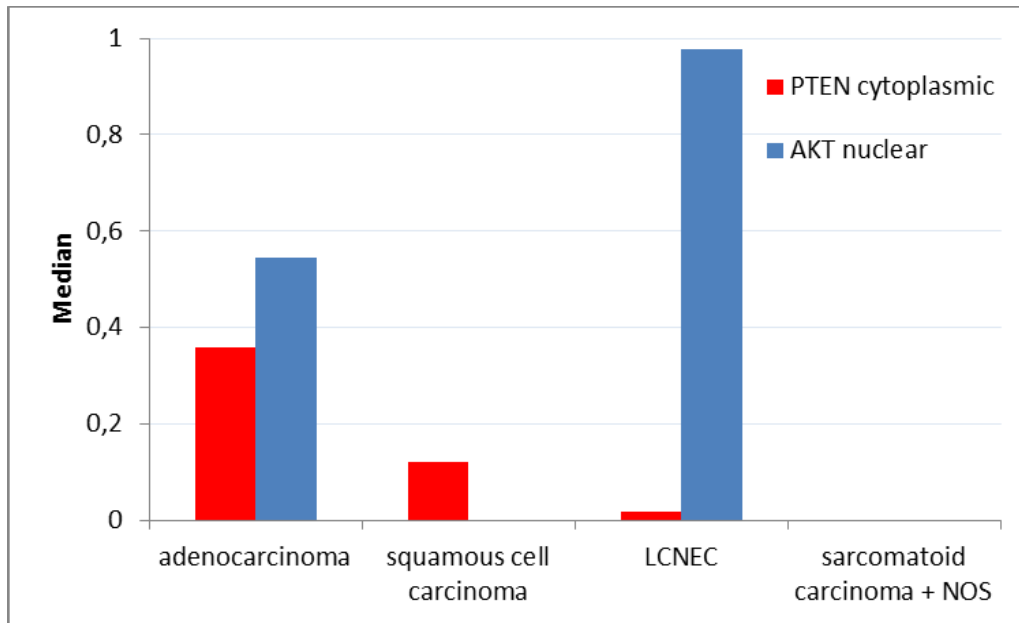


Fig. 4: Cytoplasmic PTEN and nuclear pAKT expression in the histological subtypes of NSCLC. While the cytoplasmic PTEN expression decreases, the nuclear pAKT expression increases.

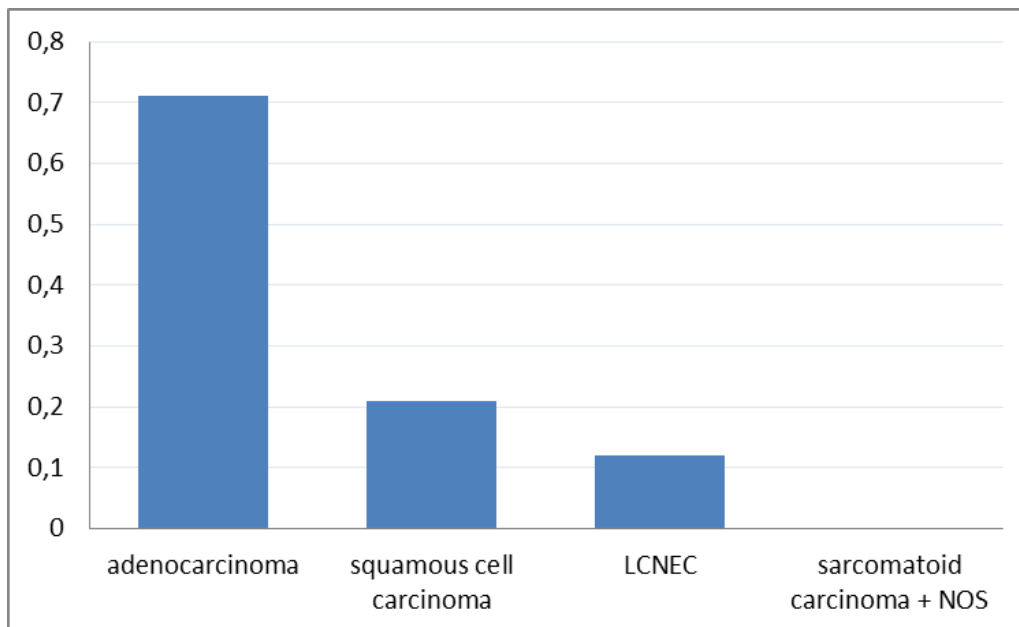


Fig.5: Co-expression of PTEN and pAKT in the histological subtypes of NSCLC. Co-expression is significantly higher in adenocarcinoma than in any other subtype ($p=0.001$).



Correlating the proteins expression with other patient characteristics PTEN shows a greater expression in the elderly (>66 yrs.; $p=0.035$) while pAKT is more pronounced in the younger patients (≤ 66 yrs.; $p=0.001$). Both proteins show a greater nuclear expression in women (PTEN $p<0.001$; AKT $p=0.047$) but no significant correlation was found with pT, pN, pM and UICC state in either one of the cell's compartments. In order to correlate patients' survival for up to 10 years with the expression of pAKT and PTEN Kaplan Meyer curves were used. pAKT shows no correlation between its expression and patient overall survival rate. PTEN on the other hand, shows a positive relation for patients with a low grade, nodal negative tumor that expresses PTEN and a longer survival compared to all other combinations ($p=0.031$; Fig.6).

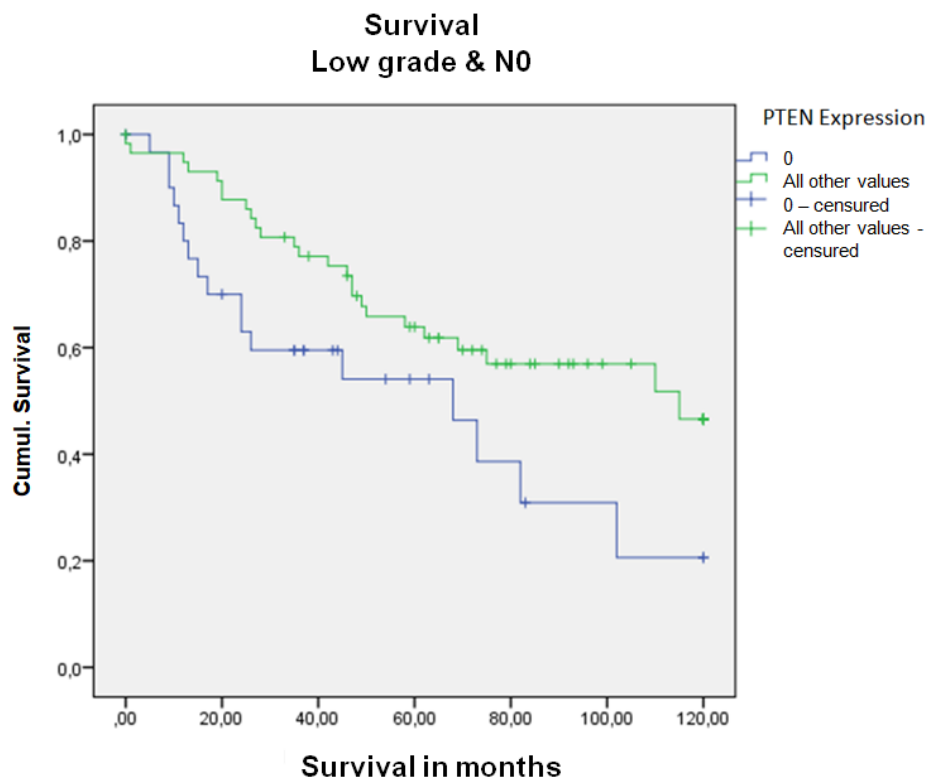


Fig. 6: Kaplan-Meier curve for the survival of patients with low grade (G1/2), nodal negative tumours in correlation with PTEN expression. 0 = no PTEN expression, all other values = PTEN expression ($p = 0.031$).



Discussion

1.3. Sub-cellular localization

Many studies have investigated PTEN and pAKT in different types of cancer for their influence in cell proliferation, tumorigenesis and survival rates with varying results. Our study emphasized on the specific location of the proteins PTEN and pAKT within the different cell compartments. While a lot of studies describe a predominant expression of PTEN in the cytoplasm, this differentiation of the sub-cellular localization is disregarded in further analyses (8, 16, 17). The importance of the localization of pAKT, especially in the nucleus, however has been described in various tumors e.g. NSCLC, endometrium and breast cancer. In these tumors the nuclear pAKT expression showed a higher prognostic value than the cytoplasmic expression (16, 18-20).

1.4. Grading

PTEN expression in our study showed an up-regulation towards poorer differentiated tumors and a significant decline in undifferentiated tumors. This loss of PTEN in high grade tumors compared to low grade tumors has also been described in other studies (8, 12, 17, 21). Cytoplasmic pAKT expression increased significantly with dedifferentiation and showed highest expression levels in undifferentiated tumors, while there was no significant correlation with nuclear expression. Reversely, in the study of endometrium carcinoma conducted by Shen et al. a correlation of nuclear pAKT expression with a dedifferentiation of the tumor was described (20). Studies concerning NSCLC and nuclear pAKT expression in relation to tumor grading have not been reported.



1.5. Histology

Our study showed high PTEN and pAKT expression in adenocarcinoma. EGFR-mutation rates in adenocarcinoma of up to 36% (22) might be responsible for the activation of the signaling pathway leading to high expression of both proteins. If PIK3CA amplification and mutation found in squamous cell carcinoma lead to an upregulation of pAKT and PTEN remains a controversy (23-25). In the poorly differentiated LCNEC, a low PTEN but the highest pAKT expression was found. The high pAKT expression could be due to pAKT activation in LCNEC (26). High mutation rates of TP53 in this tumor type (27) may lead to low PTEN and following high pAKT expression.

1.6. Patients' Survival

Our study could not find a general correlation between the expression of PTEN and pAKT and overall survival rates. Merely patients with a low grade and nodal negative tumors expressing PTEN showed a better overall survival. Studies investigating this relation present varying results. Some studies show a general correlation of PTEN expression with better survival in NSCLC (8, 21). Others describe a correlation only for adenocarcinoma or squamous cell carcinoma (16, 28). Further studies combine a positive PTEN expression with a negative pAKT expression and report a better outcome for those patients (16, 21). Similar analyses for our study population were not significant. In agreement with our findings no correlation was also found in the studies conducted by Yoshizawa et al. and Marsit et al. (29, 30). In all these studies the observation period varies between 5, 6 and up to 10 years which makes comparability difficult.

Conclusion

In conclusion, the expression of PTEN and pAKT found in our study support their close relationship within the PI3K signaling pathway. High expression of PTEN can lead to a



downregulation of pAKT. This influence on a molecular level seems to correlate closely with the different histological subtypes, their varying mutations and differentiation in NSCLC. Also the distinction between nuclear and cytoplasmic localization plays an important role and needs to be further investigated in studies concerning tumorigenesis.

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