

Creation of Mouse Models of Human Lung Cancer by Activation of PI3K/AKT-dependent Signaling.

Abstract #: 5120

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Abstract

Activation of phosphatidylinositol 3'-kinase and its downstream mediator, Akt, promotes cellular survival and induces malignant transformation. Previous studies have reported reduced expression of the PTEN lipid phosphatase, a negative regulator of AKT, and increased Ser473-phosphorylation of AKT in non-small cell lung cancer (NSCLC) biopsy samples and cell lines. Further, AKT phosphorylation is increased in biopsy samples of bronchial premalignancy, raising the possibility that AKT activation contributes to early stages of lung tumorigenesis.

We investigated the role of this pathway in lung tumorigenesis by two creating mouse models: one with conditional *PTEN* loss and the other with over-expression of an activated form of AKT (gag-AKT).

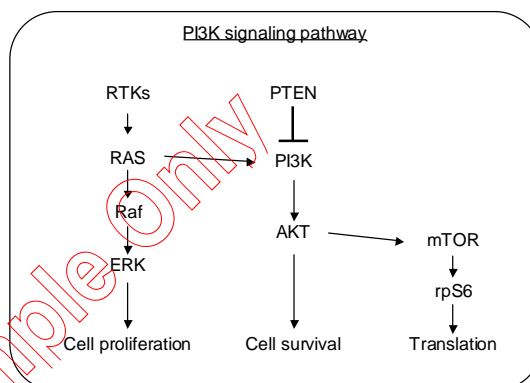
For the conditional *PTEN* model, *PTEN* was deleted by Cre-mediated recombination. Cre was expressed under the control of a bronchial epithelial-specific gene promoter (*clara cell secretory protein* or *CCSP*). By 3 months of age, *PTEN* deletion resulted in bronchial epithelial hyperplasia. Immunohistochemical analysis revealed increased Ser473 phosphorylation of Akt in hyperplastic regions.

For the gag-AKT model, expression of the gag-AKT transgene was induced by the mifepristone regulator GLP65, which was under the control of a type II alveolar cell-specific gene promoter (*surfactant protein C* or *SP-C*). Preliminary studies have shown that mifepristone treatment induced gag-AKT expression in the alveolar epithelium.

To examine whether PTEN loss acts in concert with other genetic lesions, we mated *PterfloxP* mice with LSL-K-ras^{G12D} mice, which has oncogenic *K-ras* allele under the control of a transcriptional stop codon flanked by lox sites. Preliminary results suggest that mice carrying both *PterfloxP* and LSL-K-ras^{G12D} develop lung adenocarcinoma at an accelerated rate and with more invasive features compared to mice carrying KRAS^{LSL} alone.

Introduction

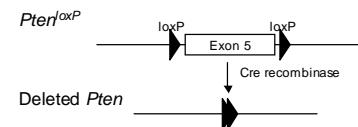
- Phosphatidylinositol 3'-kinase (PI3K) pathway is important of cell proliferation and cell survival.
- The tumor suppressor PTEN (phosphatase with tensin homology) is a negative regulator of AKT.
- PI3K pathway is activated in human lung cancers and bronchial premalignancy. (Tsao AS et al. 2003)



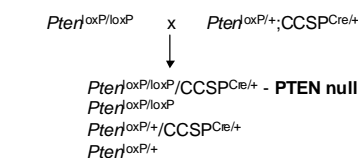
Results

Conditional *PTEN* loss model

- LoxP sequences were inserted into the endogenous *Pten* locus flanking exon 5 (Lesche R et al. 2002). Exon 5 encodes the phosphatase domain of PTEN which is deleted in many tumor-associated mutations.
- Cre was expressed under the control of CCSP (Clara cell secretory protein) gene promoter. This is a bronchial epithelial cell specific gene promoter.



- Breeding strategy



Lung-specific deletion of *Pten*

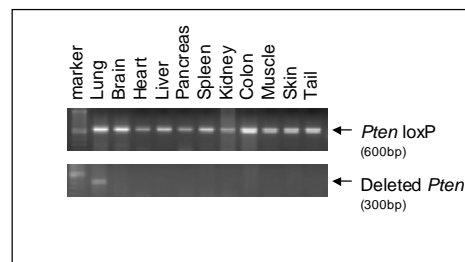


Fig. 1 PCR analysis of DNA from different tissues of *PterfloxP/CCSPCre/+* mice. *Pten* deletion is specific to lung.

Pten deletion result in bronchial epithelial hyperplasia

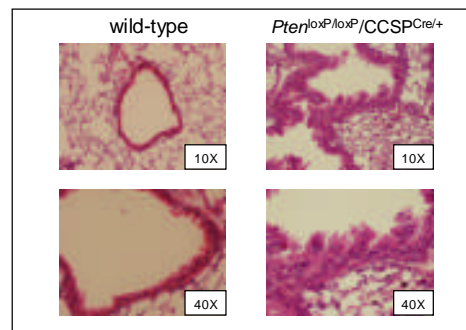


Fig. 2 Histological analysis of lung sections from wild-type mice and *PterfloxP/CCSPCre/+* mice at the age of 3 month. Hematoxylin/eosin staining shows low (Top) and high (bottom) magnification view of bronchial epithelial cells. *PterfloxP/CCSPCre/+* mice show multi layer and micro papillary formation in the bronchial epithelial cells

Pten loss increase phosphorylation of Akt in hyperplastic regions

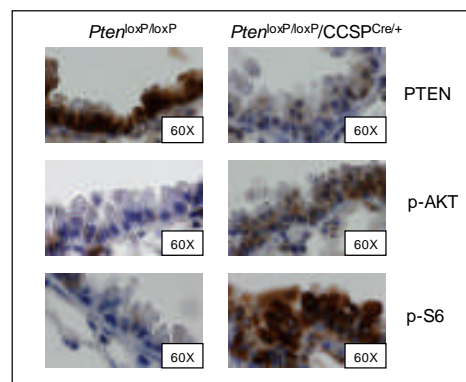
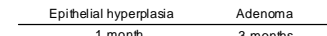


Fig. 3 Immunohistochemical analysis of PTEN, phospho-AKT^{Ser473} and phospho-S6 in the lungs of *PterfloxP/CCSPCre/+* mice and *PterfloxP/CCSPCre/+* mice. Increased Ser473 phosphorylation of AKT and S6 are observed in hyperplastic regions of *PterfloxP/CCSPCre/+* mice.

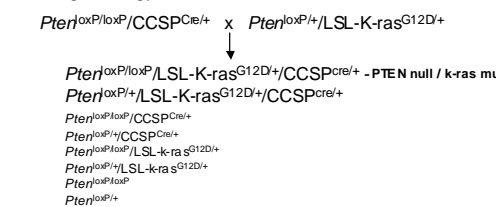
PTEN null, k-ras mutant model

- Conditional expression of oncogenic K-ras mice (LSL-K-ras^{G12D}/CCSP^{Cre/+} mice) develop lung adenoma at 3 months (Li H et al. unpublished data).



- K-ras and Pten in the ovarian surface epithelium cause endometrioid ovarian cancer (Dinulescu DM et al. 2005).
- The PI3K/AKT pathway may be important for the treatment of ras-driven human cancers (Lim KH et al. 2005).

- Breeding strategy



Pten loss and k-ras mutant mice develop lung adenocarcinoma at early time point

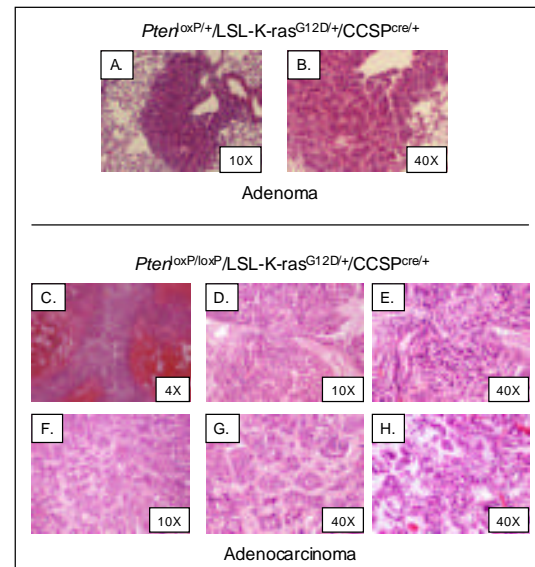


Fig. 4 Histological analysis of *PterfloxP/LSL-K-rasG12D/+CCSPCre/+* mice (A, B) and *PterfloxP/LSL-K-rasG12D/+CCSPCre/+* mice (C, D, E, F, G, H). The mice were killed at 3 month old. (A, B) Alveolar adenoma at 10X (A) and 40X (B) power. (C) Adenocarcinoma, intrabronchial spread pattern. (D, E) Invasion through bronchus at 10X (D) and 40X (E) power. (F, G) Acinar growth pattern at 10X (F) and 40X (G) power. (H) Bronchioloalveolar cell growth pattern.

gag-AKT transgenic model

- gag-AKT protein is a fusion protein, that viral gag protein fused akt protein (Jones RG et al. 2000).



- Expression of the gag-AKT transgene is induced by the Mifepristone (RU486) regulator GLP65, which is under the control of a type II alveolar cell-specific gene promoter (*surfactant protein C* or *SP-C*) (Zhao B et al. 2001).

- gag-AKT mice were bred with SPC-p65 mice to generate bitransgenic mice.

RU486 inducible expression of gag-AKT in bitransgenic mice.

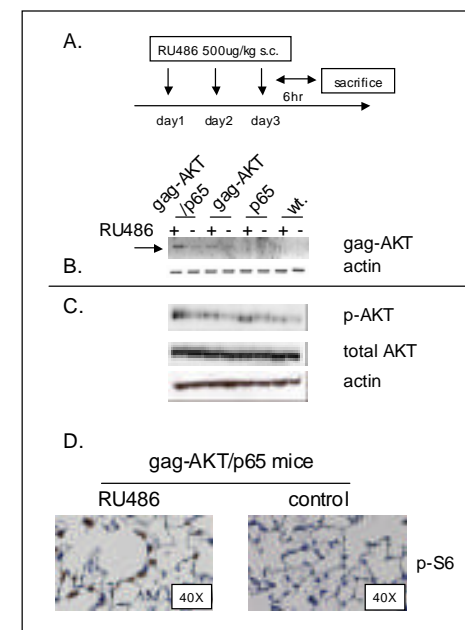


Fig. 5 The mice received treatment with RU486 or sesame oil (control) for 3 days. (A) RT-PCR of gag-AKT mRNA and (B) Western blot analysis of phospho-AKT^{Ser473}, total akt and actin protein expression in the lungs after treatment on bitransgenic mice (gag-AKT/p65), monotransgenic mice (gag-AKT or p65) and wild type mice (wt.). (C) Immunohistochemical analysis of phospho-S6 in the lungs of gag-akt/p65 bitransgenic mice.

Summary

- 1) The PTEN null mice show increased phosphorylation of AKT and its downstream mediator, S6.
- 2) Bronchial specific PTEN deletion caused hyperproliferative lesions in the bronchial epithelia at 3 months.
- 3) PTEN loss accelerated and enhanced invasive properties of lung adenocarcinoma induced by oncogenic K-ras.
- 4) Ligand inducible lung specific expression of gag-AKT increase phosphorylation of AKT and S6 in the alveolar cells.
- 5) These models may be useful in defining the role of PI3K/AKT alone and in combination with other genetic lesions in lung tumorigenesis.

References

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