Inactivation of PHLDA3 gene leading to tumorigenesis of PNETs, its molecular mechanisms and predicting prognosis

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Background and Aim:

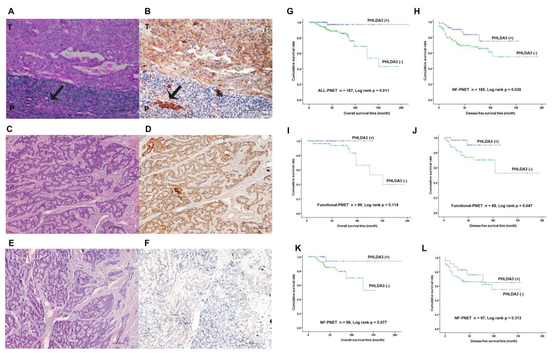
- Previous studies (including ours) showed that loss of heterozygosity (LOH) at chromosome 1q31 (*PHLDA3* gene located) frequently occurred in sporadic insulinoma, gastrinoma and NF-PNETs, respectively [1-3]. Inactivation of PHLDA3 resulted in hyperplastic islets in PHLDA3-deficient mice, however, PNETs development was not found [3].
- Aim: to demonstrate the role of *PHLDA3* gene in the tumorigenesis of PNETs and assess its prognostic value in patients.

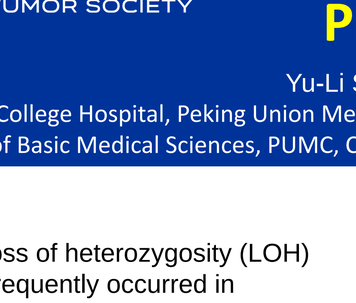
Methods:

- Expression of PHLDA3 protein was examined in PNETs specimens by immunohistochemistry and correlated with survival of patients.
- Methylation of *PHLDA3* gene was tested by methylation specific PCR.
- Microsatellite markers are used to detect *PHLDA3* LOH.
- *PHLDA3* gene was knocked out in rats by CRISPR-Cas9.
- INS-1 and QGP-1 cell lines were transfected with *PHLDA3* gene.
- The proliferation and apoptosis were determined by the MTT assay and TUNEL assay, flow cytometry, respectively, in two PNET cell lines transfected with *PHLDA3* gene.
- $P \leq 0.05$ was considered as significance.

Results:

1. Reduced expression of PHLDA3 was correlated with poorer survival in patients





2. LOH were found in 8 of 33 PNETs. Only one of 8 tumors with LOH expressed PHLDA3 protein (12%), while 13 of 25 tumors without LOH expressed PHLDA3 (52%)

References

1, Yang Y, Liu T, Chen Yuan-Jia et al. Int. J Cancer 2005; 117: 234-240

2, Chen Yuan-Jia, et al. Cancer Res. 2003; 63, 817–823

3, Ohki R., et al. Proc Natl. Acad. Sci. 2014; 111: E2404-2413

Conclusions

- PNETs developed in PHLDA3-deficient rats.
- A two-hit inactivation of the *PHLDA3* gene could result in tumorigenesis in part of human PNETs and correlated with unfavorable prognosis.

Emphasise Important Words

PHLDA3, PHLDA3-deficient rat, tumorigenesis of PNETs, PHLDA3 methylation and LOH, apoptosis, prognosis

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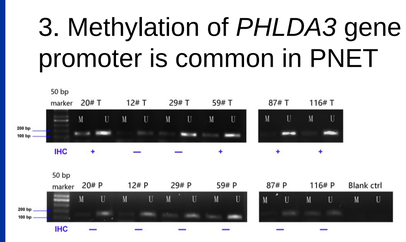
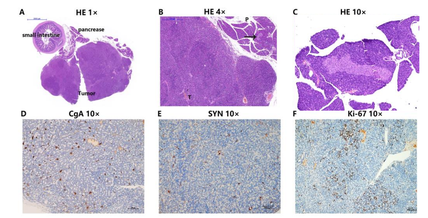
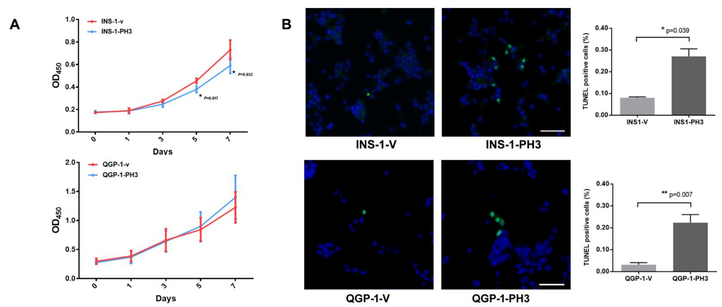
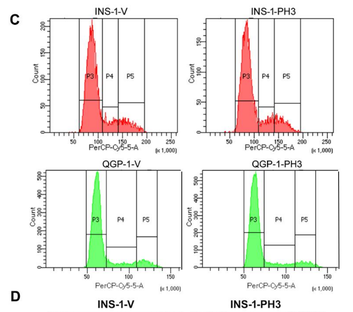
3. Methylation of *PHLDA3* gene promoter is common in PNET



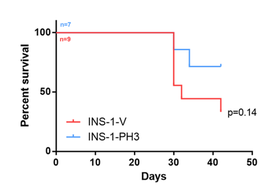


4. PNET and hyperplastic islets were seen in PHLDA3-deficient rats (one islet was even more that 1 mm) but no tumor was found in control group.





5. Overexpression of PHLDA3 in rat insulinoma cell line (INS-1) significantly promoted apoptosis of tumor cells (P=0.039) and inhibited cell growth but did not influence on cell cycle, migration. Overexpression of PHLDA3 in human QGP-1 cell line also promoted tumor cell apoptosis (P=0.007).



6. Survival time of nude mice with overexpression of PHLDA3 PNET seems longer than the control group (n = 16, log rank P =0.140).

Future Directions for Research:

- To further clarify the mechanism of PHLDA3 promoted apoptosis.