RESEARCH ARTICLE

Molecular Evolution of PTEN Pseudogenes in Mammals

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Abstract

Phosphatase and tensin homolog (PTEN) is a tumor-suppressor gene. PTEN pseudogene (PTENp) acts as an endogenous RNA, which regulates its parental gene by competitively binding to the 3' UTR of PTEN gene in the human. Despite the importance of this pseudogene, little is known about the molecular evolution of PTENp in mammals. In this study, we identified 37 pseudogenes from 65 mammalian genomes. Among them, 32 were from rodents or primates. Phylogenetic analysis showed a complex evolutionary history of this gene family. Some PTENps were shared both in primates and rodents. However, some PTENps were shown to be species-specific, such as the tasmanian devil PTENp1, nine banded armadillo PTENp1 and gibbon PTENp1. Most interestingly, the naked mole rat (NMR), an anticancer model organism, possessed 17 copies of PTENps, which were classified into four clades based on the phylogenetic analyses. Furthermore, we found that all the 3'UTR of PTEN and PTENps shared common microRNA (MicroRNA) binding sites in NMR, based on our prediction of specific MicroRNA binding sites. Our findings suggested that multiple gene duplications have occurred in the formation of PTEN/PTENp gene family during the evolution of mammals. Some PTENps were relatively ancient and were shared by primates and rodents; others were newly originated through species-specific gene duplications. PTENps in NMR may function as competitive endogenous RNAs (ceRNAs) to regulate their counterpart genes by competing for common MicroRNAs, which may be one of the interpretations for the cancer resistance in NMR.

Introduction

In 1977, Jacq et al found a truncated version of the 5S ribosome DNA in Xenopus laevis, which is homologous to the native gene, and this fragment of genomic sequences was first named Pseudogene[1]. Traditionally, pseudogenes were defined as the functionless relatives of protein-coding genes, mainly due to the presence of premature stop-codons or frame shifts, and have long been viewed as the non-functional genomic remnants during evolution[2]. Based on their formation mechanisms, pseudogenes can be classified into three categories, which are unitary pseudogenes, unprocessed pseudogenes, and processed pseudogenes. Unitary pseudogenes, previously referred to those functionless genes, originated from functional genes by
various mutations. Unprocessed pseudogenes are derived directly from duplications of DNA sequences, with their original intron-exon structures and promoter elements having been maintained. Processed pseudogenes are formed by retrotransposition of mRNA transcripts. Introns and other regulatory elements such as enhancers and promoter elements have been lost during the process of pseudogenization.

It is proposed that messenger RNA, transcribed pseudogenes, and long non-coding RNAs can crosstalk by competing for common MicroRNAs[3,4]. These RNA transcripts were termed as competitive endogenous RNAs (ceRNAs). The activity of ceRNAs forms a large-scale regulatory network across the transcriptome. More and more experimental evidences, such as PTEN-PTENP1[5], TUSC2-TUSC2P[6], HMGA1-HMGA1P[7], CYP4Z1-CYP4Z2P[8] and BRAF-BRAFP1[9], support the ceRNA regulation hypothesis. For example, PTEN negatively regulates intracellular levels of phosphatidylinositol-3,4,5-trisphosphate in cells and acts as a tumor suppressor by negatively regulating Akt/PKB signalling pathway[10]. The PTEN pseudogene (PTENp1) is a processed pseudogene, which shows high sequence similarity with PTEN in human. The binding sites of the MicroRNAs, including miR-20a, miR-19b, miR-21, miR-26a and miR-214, are highly homologous in the 3’UTR of PTEN and PTENp1, and those MicroRNAs are able to regulate the translation of PTEN in humans[5]. PTENp1 can thus regulate PTEN by competitively binding to these MicroRNAs, and serving as decoy for PTEN-related MicroRNAs. Furthermore, decreasing of the copy number of PTENp1 was observed in sporadic colon cancer, which was correlated with a decrease of PTEN, thus leading to the proposal that PTENp1 is a bona fide tumour suppressor gene[5]. In addition, Johnsson et al. reported that PTENp1-expressed transcripts can also act as antisense RNAs (asRNAs) to regulate PTEN expression at both transcriptional and post-transcriptional levels[11]. PTENp1 encoded two asRNA isoforms: PTENp1 asRNA alpha and beta. The alpha isoform acts as a negative regulator for transcription of PTEN. Because of the sequence homology, PTENp1 asRNA alpha recruits the DNA methyltransferase 3a (DNMT3a) and Enhancer of Zeste Homolog 2 (EZH2) to the PTEN promoter, resulting in PTEN transcription suppressed by the formation of H3K27me3. In contrast, the beta isoform forms RNA-RNA interactions with PTENp1 sense transcript. This RNA-RNA interaction stabilizes PTENp1 sense, consequently affecting MicroRNA sequestration and ultimately PTEN protein level.

Except for PTENp1, some other pseudogenes were reported to perform as ceRNAs. The tumour suppressor candidate-2 gene pseudogene (TUSC2P) can talk with the TUSC2 gene through MicroRNA response elements (MREs), as well as PTEN-PTENP1. The 3’UTR of TUSC2P captures these TUSC2-targeting MicroRNAs, which increases the translation of TUSC2 and then inhibits cell proliferation[6]. In addition, Esposito and co-workers found seven pseudogenes homologous to the high mobility group AT-hook 1 (HMGA1) gene, which is associated with insulin resistance and carcinogenesis[7]. Two of them, the HMGA1P6 and HMGA1P7, showed high sequence similarity with each other and conserved MRE with the parental gene. HMGA1P6 and HMGA1P7 also act as ceRNAs by competitively binding to MicroRNAs with the HMGA1, regulating the expression of HMGA1 and accordingly increasing proliferation and cell migration[7]. Florian et al. discovered that BRAFP1 functions as a ceRNA of BRAF in humans and mice, competing for miR-134, miR-543, miR-653, miR-30a, miR-182 and miR-876[9]. Most interestingly, the effect of over-expression of the 3’UTR of BRAFP1 was more significant than over-expression of its CDS on the parental gene expression and proliferation[9]. Overall, these findings suggest that 3’UTRs from both pseudogenes and coding genes may possess powerful biological activity through their ability to act as endogenous decoys for MicroRNAs.

Despite the importance of those functional pseudogenes, their evolutionary histories were largely unknown. In this study, we investigated the molecular evolution of PTEN/PTENp gene
family in mammals. By searching the available mammalian genome sequences, we found 37 pseudogenes from 65 mammalian genomes. Most intriguingly, we identified 17 copies of \textit{PTEN}ps from naked mole rat (NMR), an anticancer model organism, and found that all of these genes shared common MicroRNA binding sites with their PTEN gene, suggesting that the \textit{PTEN}ps in NMR may be functional in regulating their cognate genes by competing for MicroRNA binding sites, just as that found in the humans.

Materials and Methods

Our animal experiment was approved by the Institutional Animal Care and Use Committee of the Sichuan Agricultural University under permit number DKY- B20150301

Sequences obtain

The \textit{PTEN} mRNA sequences from 65 mammals were downloaded from National Centre for Biotechnology Information (NCBI) GenBank, and their \textit{PTEN}ps were identified by BLAST, the reference genomic sequences database, using \textit{PTEN} mRNA as query. All potential pseudogenes meet at least one of the following three criterions: 1. incomplete open reading frame (ORF), 2. frame-shifts and 3. premature stop codons. All were labelled as pseudogenes in GenBank.

Phylogenetic analyses

As different regions of a gene play different roles and are, apparently, subjected to different stringencies of functional constraints, it has been customary to treat different regions separately. In contrast to the coding regions of genes, the rates in non-coding regions are usually higher. Furthermore, most of them vary greatly in the length of these noncoding regions. For example, the length of 3’UTR of \textit{PTEN}/\textit{PTEN}ps in primates are larger at 1000bp, but in most of other species are less than 1000bp. This variation makes the phylogenetic analyses using non-coding regions very difficult. Therefore, in this study, we only compared the evolutionary rate of CDS of \textit{PTEN}/\textit{PTEN}ps. The CDS region of \textit{PTEN} and \textit{PTEN}p sequences of mammals were aligned using ClustalW in BioEdit\cite{12} followed by manual adjustments. Maximum Likelihood (ML), Maximum Parsimony (MP) and Neighbour Joining (NJ) phylogenetic trees were conducted by using MEGA6.0\cite{13}. Fourteen sequences out of 102 identified \textit{PTEN}s and \textit{PTEN}ps were removed in the phylogeny analyses because of too many ambiguous bases, long gaps or the incompleteness of the sequences. The removed sequences were degu \textit{PTEN}p1, NMR\textit{PTEN}p12, NMR \textit{PTEN}p15, NMR \textit{PTEN}p16, horse \textit{PTEN}, orangutan\textit{PTEN}p1 and chimpanzee \textit{PTEN}p1 (these sequences contained ambiguous bases); cattle \textit{PTEN}, duckbill platypus \textit{PTEN}, domestic water buffalo \textit{PTEN}, southern American pika\textit{PTEN} and orguinea pig \textit{PTEN}p1 (these sequences showed big gaps); guinea pig \textit{PTEN} and European domestic ferret \textit{PTEN} (these sequences were incomplete). And then the coding regions of 88 sequences were used for phylogenetic tree construction. In addition, a dataset contains 67 sequences from Primate, Rodents, Even-toed ungulates, Carnivores, Cingulata and Dasyuromorphia, where both \textit{PTEN} and \textit{PTEN}ps were identified, was also used to construct phylogenetic trees. Kimura 2-parameter method\cite{14} were used to infer NJ tree implemented in the program MEGA6.0. For ML tree, Tamura 3-parameter model with a discrete gamma distribution was used as suggested by MEGA6.0. Default settings in MEGA6.0 were used in reconstructing the MP tree. For ML, MP and NJ methods, 1000 bootstrap replications were conducted to evaluate the reliabilities of the reconstructed trees.
MicroRNAs binding sites Prediction

MicroRNA binding sites of the 3' UTR of PTENs and PTENps were predicted by PITA algorithm[15]. Five MicroRNAs (mir-19b, mir-20a, mir-21, mir-26a, mir-214), which can competitively bind with PTEN and PTENp1 in human[5], were downloaded from miRBase database[16]. Then the specific MicroRNAs and 3’ UTR of PTENs or PTENps were uploaded to the Online microRNA prediction tool to predict MicroRNA binding sites[15]. Minimum seed size is set to 6 and other parameters were as default settings. ΔΔG is an energetic score, the lower (more negative) its value, the stronger the binding of the MicroRNA to the given site is expected. We first calculated the ΔΔG values for the known binding pairs of MicroRNA and the corresponding binding sites in the humans, and then conservatively set the lowest value-3.8 as our cut-off value in this study.

Results

Pseudogene Sequences

In total, we found 65 functional genes and 37 pseudogenes from 65 genomes of mammals by BLAST using PTEN mRNA as query (S1 and S2 Tables). We found that 17 out of 65 species possess one or more copies of PTENps. Among them, 32 out of 37 pseudogenes identified in this study were from primates and rodents. We identified 9 species each possessed one PTENp in primate. Interestingly, these PTENps only existed in old world monkeys and hominoids. Five species of rodents were found to possess PTENps. And most excitingly, 17 copies of PTENps in NMR were identified (Table 1). In addition, one copy of PTENp was found in the nine banded armadillo and Tasmanian devil. Three copies of PTENps were found in the pig.

Phylogenetic analyses

To explore the evolutionary relationships of these PTENps and PTEN genes in mammals, we constructed the phylogenetic trees based on the coding region of 88 sequences using the Neighbour joining (NJ)[17](S1 Fig), Maximum parsimony (MP)[18](S2 Fig) and Maximum Likelihood (ML) methods (S3 Fig) separately. To make the result more clear, we removed sequences from those orders where no PTEN pseudogene was found. Based on the coding region of the remaining 67 sequences, we constructed the NJ (Fig 1), MP (S4 Fig) and ML tree (S5 Fig). All trees showed overall similar topology. In these trees, PTENps were dispersed into several clades rather than forming one clade, suggesting that multiple gene duplications have occurred during the evolution of PTEN/PTENp gene family (Fig 1 and S1–S5 Figs). As showed in Fig 1, some PTENps existed for a relatively long time such as clade 1, which was shared by the NMR and the pig; and clade 9, which was shared by species from primates and rodents. In addition, the two clades showed longer branch lengths compared to other clades of PTENps, which indicated that PTENps of the two clades were relatively old. However, some PTENps were relatively young. For example, clade 2, clade 4 and clade 6 displayed a species-specific evolutionary pattern, in which PTENp clustered with its cognate gene, suggesting these PTENps emerged after the divergences of these species from their sister groups. What’s more, we found that the branch lengths of PTENps were longer than that of the PTEN, suggesting faster evolutionary rate of CDS of PTENps than the PTENs in mammals (Fig 1). PTENps in NMR were divided into four clades in the phylogenetic tree. Clade 1 includes PTENp17, PTENp8 and PTENp4. Clade 7 contains only PTENp9. Clade 8 includes PTENp1, PTENp2, PTENp3, PTENp5, PTENp6 and PTENp7. Clade 9 includes PTENp10, PTENp11, PTENp13 and PTENp14. PTENps in clade 8 were NMR specific with shorter branch lengths, suggesting that these genes appeared recently.
### Table 1. The number of **PTENs** and **PTENps** in mammals.

| Order               | Common name           | Scientific name          | No. of PTEN | No. of PTENp |
|---------------------|-----------------------|--------------------------|-------------|--------------|
| **Primates**        |                       |                          |             |              |
| Primates            |                       |                          |             |              |
| Human               | Homo sapiens          | 1                        | 1           |              |
| Chimpanzee          | Pan troglodytes       | 1                        | 1           |              |
| Pygmy chimpanzee    | Pan paniscus          | 1                        | 1           |              |
| Orangutan           | Pongo abelii          | 1                        | 1           |              |
| Gorilla             | Gorilla gorilla gorilla | 1                       | 1           |              |
| Crab-eating macaque | Macaca fasicularis    | 1                        | 1           |              |
| Rhesus macaque      | Macaca mulatta        | 1                        | 1           |              |
| Baboon              | Papio anubis          | 1                        | 1           |              |
| Green monkey        | Chlorocebus sabaeus   | 1                        | NO          |              |
| Marmoset            | Callithrix jacchus    | 1                        | NO          |              |
| Squirrel monkey     | Saimiri boliviensis   | 1                        | NO          |              |
| Gibbon              | Nomascus leucogenys   | 1                        | 1           |              |
| Bush baby           | Otomlemur garnetti    | 1                        | NO          |              |
| **Rodents**         |                       |                          |             |              |
| Rodents             |                       |                          |             |              |
| Mouse               | Mus musculus          | 1                        | NO          |              |
| Rat                 | Rattus norvegicus     | 1                        | NO          |              |
| Naked mole rat      | Heterocephalus glaber | 1                        | 17          |              |
| Blind mole rat      | Nannospalax gallii    | 1                        | 1           |              |
| Golden hamster      | Mesocricetus auratus  | 1                        | NO          |              |
| Chinese hamster     | Cricetulus griseus    | 1                        | NO          |              |
| Prairie vole        | Microtus ochrogaster  | 1                        | NO          |              |
| Prairie deer mouse  | Peromyscus maniculatus bairdi | 1    | NO          |              |
| Guinea pig          | Cavia porcellus       | 1                        | 1           |              |
| Lesser egyptian jerboa | Jaculus jaculus    | 1                        | NO          |              |
| Degu                | Octodon degus         | 1                        | 3           |              |
| Long-tailed chinchilla | Chinchilla lanigera | 1                        | 1           |              |
| Thirteen-lined ground squirrel | Spermophilus tridecemlineatus | 1    | NO          |              |
| **Even-toed ungulates** |                   |                          |             |              |
| Even-toed ungulates |                       |                          |             |              |
| Bovine              | Bos taurus            | 1                        | NO          |              |
| Wild yak            | Bos mutus             | 1                        | NO          |              |
| Goat                | Capra hircus          | 1                        | NO          |              |
| Sheep               | Ovis aries            | 1                        | NO          |              |
| Chiru               | Partholops hodgsonii  | 1                        | NO          |              |
| Domestic water buffalo | Bubalus bubalis   | 1                        | NO          |              |
| Killer whale        | Orcinus orca          | 1                        | NO          |              |
| Sperm whale         | Physeter catodon      | 1                        | NO          |              |
| North Pacific minke whale | Balaenoptera acutorostrata scammoni | 1 | NO | |
| Pig                 | Sus scrofa            | 1                        | 3           |              |
| Alpaca              | Vicugna pacos         | 1                        | NO          |              |
| Wild bactrian camel | Camelus ferus         | 1                        | NO          |              |
| **Carnivores**      |                       |                          |             |              |
| Carnivores          |                       |                          |             |              |
| European domestic ferret | Mustela putorius furo | 1                        | NO          |              |
| Dog                 | Canis lupus familiaris| 1                        | NO          |              |
| Cat                 | Felis catus           | 1                        | NO          |              |
| Siberian tiger      | Panthera tigris altaica | 1                      | NO          |              |
| Weddell seal        | Leptonychotes weddellii| 1                      | NO          |              |
| Pacific walrus      | Odobenus rosmarus divergens | 1 | NO | |
| Giant panda         | Ailuropoda melanoleuca | 1                        | NO          |              |

(Continued)
MicroRNA binding sites prediction

To investigate whether 3’UTR of these PTENps could potentially bind to specific MicroRNAs just like in the human, we used the PITA algorithm to predict the binding sites of specific MicroRNAs on 3’UTR of PTENps. We chose the PITA for MicroRNA target prediction because it has high prediction accuracy and low false positive rate, since it pays more attention to the accessibility but not the conservation of the target sequences[15,19]. It was evidenced that 5 MicroRNAs (mir-19b, mir-20a, mir-21, mir-26a, mir-214) could bind to the 3’UTR of PTEN and PTENp1, and thus act as MicroRNA sponges to protect their parent gene from MicroRNA disturbance in the human[5]. In this study, we predicted the binding sites of these five MicroRNAs in 3’UTR of PTEN and PTENp1 identified in this study (S3 and S4 Tables). Interestingly, we found that the 3’UTR of PTENp and PTEN shared MicroRNAs bind sites in most cases, except for the PTENp1 in the blind mole rat (BMR), in which no shared binding site was found. This result suggested that MicroRNAs were potentially able to bind to the 3’UTR of both PTENps and their cognate PTENs. Most importantly, mir-19b existed in all of 3’UTR of PTENs and NMR (Table 2). In addition, the NMR had eight copies of PTENps shared three different kinds of MicroRNAs, and four copies of PTENps shared two sorts of MicroRNAs. MicroRNA binding sites identified in this study are illustrated in Fig 2.

Discussion

In this study, we found that PTENps not only existed in the human, but also appeared in some species of primates, rodents, even-toed ungulates, carnivores, cingulata and dasyuromorphia, suggesting that PTENps emerged before the divergences of these mammalian orders. However, the majority of other mammals (48 out of 65 mammals) lacked the PTENp, which may be due to either the loss of PTENps during evolution, or the pseudogenization of PTEN never took
Fig 1. The Neighbour Joining tree of 67 PTENs and PTENps in mammals. All bootstrap values were showed. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances. Clade 1 to clade 9 were tagged by red square.

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place in these species. Since no clade of PTENp genes was shared by all mammalian orders, providing no evidence supporting the origination of PTENp before divergence of mammals, it is not clear whether PTENps were lost in these 48 species. According to the sequences alignment, we observed that some PTENps are completely duplicated from their parental gene and some are partially duplicated, such as the NMR PTENp10, 11, 13 and 14. In addition, we found some PTENps showed species-specific evolutionary pattern, such as the Tasmanian devil PTENp1, nine banded armadillo PTENp1 and gibbon PTENp1. These results suggest that the mammalian PTENps were originated by multiple gene duplications, and experienced the so called ‘birth and death’ evolution[20].

Table 2. The shared specific miRNAs of PTENs and PTENps 3’UTR.

| Name of PTEN          | Name of Pesudogene | PTEN and PTENp 3’UTR shared miRNAs                   |
|-----------------------|--------------------|-----------------------------------------------------|
| Human PTEN            | Human PTENp1       | miR-20a,miR-21,miR-214,miR-19b,miR-26a              |
| Chimpanzee PTEN       | Chimpanzee PTENp1  | miR-20a,miR-21,miR-214,miR-19b,miR-26a              |
| Pygmy chimpanzee PTEN | Pygmy chimpanzee PTENp1 | miR-20a,miR-21,miR-214,miR-19b,miR-26a |
| Gorilla PTEN          | Gorilla PTENp1     | miR-19b,miR-26a                                      |
| Baboon PTEN           | Baboon PTENp1      | miR-20a,miR-21,miR-214,miR-19b,miR-26a              |
| Rhesus macaque PTEN   | Rhesus macaque PTENp1 | miR-20a,miR-21,miR-214,miR-19b,miR-26a |
| Crab eating macaque PTEN | Crab eating macaque PTENp1 | miR-20a,miR-21,miR-214,miR-19b,miR-26a |
| Gibbon PTEN           | Gibbon PTENp1      | miR-20a,miR-21,miR-214,miR-19b,miR-26a              |
| Blind mole rat PTEN   | Blind mole rat PTENp1 | no shared miRNA                                    |
| Naked mole rat PTEN   | Naked mole rat PTENp1 | miR-20a,miR-19b,miR-26a                           |
| Pig PTEN              | Pig PTENp1         | miR-20a,miR-19b                                     |
| Pig PTENp2            | miR-20a,miR-19b    |                                                     |
| Pig PTENp3            | miR-20a,miR-19b    |                                                     |
| Tasmanian devil PTEN  | Tasmanian devil PTENp1 | miR-20a,miR-21,miR-19b,miR-26a            |
| Nine banded armadillo PTEN | Nine banded armadillo PTENp1 | miR-20a,miR-21,miR-214,miR-19b,miR-26a |

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Interestingly, 17 copies of PTENp were identified in NMR, which had a high resistance to tumours[21]. Our further MicroRNA binding site prediction results showed that some MicroRNAs can bind with both PTENs and PTENps in the NMR and other mammals (Table 2). And most excitingly, we found conserved binding sites for the mir-19b, mir-20a and mir-26a in the
3’UTR of most of the NMR $PTEN_{ps}$ and the $PTEN$. Thus, it is possible that the $PTEN_{ps}$ act as the ceRNAs to regulate the $PTEN$ expression in the NMR as well as in the human. A recent study by Abegglen et al. proposed that multiple copies of $TP53$ genes in the elephant may help this large animal in resisting cancer[22]. Similarly, the multiple copies of $PTEN_{ps}$ in the NMR may also contribute to its unusual resistance to cancer. But further studies on the expression of $PTEN_{ps}$ and the interaction with miRNAs are needed to support this hypothesis.

However, the BMR, which showed a striking resistance to cancer as well as the NMR[23], is different from the NMR in terms of the copy numbers of $PTEN_{ps}$ and the MicroRNA binding sites. First, we only found one $PTENp$ in the BMR compared to 17 copies of it in the NMR. Second, unlike in the NMR, no common MicroRNA binding sites were predicted in the 3’UTR of $PTENp_1$ and its $PTEN$ in BMR. This may indicate that the anticancer mechanism in the BMR is different from that of the NMR. However, the high cut-off value we set may lead to no shared MicroRNA binding sites was found in this study, and it was also possible that the gene-pseudogene crosstalk was mediated by different MicroRNAs in the two species. In addition, some other factors also showed differences between these two species. For example, Fang et al determined that BMR have evolved a cancer-resistance mechanism depending on heightened immunoinflammatory response via gene amplification within the interferon-β1 pathway[23]. But in another study, Tian et al suggested that NMRs had evolved a higher concentration of high-molecular-mass hyaluronan (HA) that restricted cell division when cells gathered closely resulting in cancer resistance[21]. Hence, it is possible that the multiple $PTEN_{ps}$ in NMR function as ceRNAs to regulate its cognate gene by competing for common MicroRNAs, may play an important role in anticancer. Keep in mind, this mechanism may not fit for BMR.

**Conclusions**

In conclusion, our findings established that the $PTEN_{ps}$ in mammals originated by multiple gene duplications and experienced the ‘birth and death’ evolution pattern. Some $PTEN_{ps}$ have existed for a long time whereas others have appeared recently. $PTEN_{ps}$ may function as ceRNAs to regulate their $PTEN$ in mammals. Interestingly, the multiples of $PTEN_{ps}$ may compete for the common MicroRNA binding sites in the NMR as well as in the human, which may be responsible for the anticancer trait. These results provide a possible explanation for this anticancer model. However further experiments are needed to prove this hypothesis.

**Supporting Information**

**S1 Fig. The Neighbour Joining tree of 88 $PTEN$s and $PTEN_{ps}$ in mammals.** All bootstrap values were showed.
(ESP)

**S2 Fig. The Maximum Parsimony tree of 88 $PTEN$s and $PTEN_{ps}$ in mammals.** All bootstrap values were showed.
(ESP)

**S3 Fig. The Maximum Likelihood tree of 88 $PTEN$s and $PTEN_{ps}$ in mammals.** All bootstrap values were showed.
(ESP)

**S4 Fig. The Maximum Parsimony tree of 67 $PTEN$s and $PTEN_{ps}$ in mammals.** All bootstrap values were showed.
(ESP)
S5 Fig. The Maximum Likelihood tree of 67 PTENs and PTENps in mammals. All bootstrap values were showed. (EPS)

S1 Table. The detail information about PTENs and PTENps in mammals. (XLSX)

S2 Table. The detail information about PTENps in mammals. (XLSX)

S3 Table. The name and sequence information of specific miRNAs. (XLSX)

S4 Table. The prediction miRNA binding sites of PTENs and PTENps 3’UTR in mammals by PITA (The cutoff value is ddG<-3.81). (XLSX)

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Methodology: JT.
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Supervision: YL.
Validation: YL.
Visualization: JT.
Writing – original draft: JT.
Writing – review & editing: YL JT.

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