



LETTER TO THE EDITOR

Open Access

# The importance of distinguishing pseudogenes from parental genes

Luke B Hesson\* and Robyn L Ward

## Dear Editor,

The July-August 2014 issue of *Clinical Epigenetics* featured a research article describing *PTEN* promoter hypermethylation in multiple myeloma by Piras *et al.* [1]. The importance of the *PTEN* gene has resulted in significant efforts to identify sequence, expression and methylation changes in cancer. Piras *et al.* concluded that *PTEN* hypermethylation occurred in a subset of multiple myeloma cases but that hypermethylation did not correlate with reduced gene expression or clinical parameters. The *PTEN* mRNA shares 97.8% sequence identity with a pseudogene known as *PTENP1*. A 921-bp region of the promoters of these genes is also 91% identical. Consequently, careful consideration of assay design is required to avoid amplification of *PTENP1* rather than *PTEN* sequences. However, the method used by Piras *et al.* for measuring *PTEN* mRNA did not distinguish between these homologues, despite numerous studies showing that *PTENP1* mRNA is ubiquitously expressed in both normal and cancer specimens [2-5]. Furthermore, previous studies have demonstrated that apparent methylation of the *PTEN* promoter is likely attributable to the non-specific amplification of the highly homologous *PTENP1* gene [6,7]. We have shown that the only reliable method for distinguishing between *PTEN* and *PTENP1* promoter methylation is single-molecule bisulfite sequencing that utilizes sequence differences between the two genes to separately analyze individual promoter molecules [6,8]. These methodological challenges make comparisons between methylation and expression impossible when using assays that do not reliably discriminate between *PTEN* and *PTENP1*, and also negate the value of correlating these features with clinicopathological characteristics.

The challenges posed by sequence homology with pseudogenes are by no means particular to the *PTEN* gene. For example, the DNA mismatch repair gene *PMS2* shares >95.2% sequence identity with at least six other

genes (*PMS2CL*, *PMS2L2*, *PMS2P4*, *PMS2P5*, *PMS2P1* and *PMS2P11*) making analysis of the *PMS2* CpG island promoter region particularly challenging.

In light of the recent manuscript by Piras *et al.*, it is necessary to highlight the importance of rigorous methodology when investigating DNA methylation changes in cancer, especially concerning genes with homologues or pseudogenes such as *PTEN*.

## Competing interests

The authors declare that they have no competing interests.

Received: 28 November 2014 Accepted: 28 November 2014

Published online: 31 December 2014

## References

1. Piras G, Monne M, Palmes a, Calvisi A, Asproni R, Vacca F, Pilo L, Gabbas A, Latte G: Methylation analysis of the phosphatase and tensin homologue on chromosome 10 gene (PTEN) in multiple myeloma. *Clin Epigenetics* 2014, **6**:16.
2. Fujii GH, Morimoto AM, Berson AE, Bolen JB: Transcriptional analysis of the PTEN/MMAC1 pseudogene, psiPTEN. *Oncogene* 1999, **18**:1765-1769.
3. Ioffe YJ, Chiappinelli KB, Mutch DG, Zigelboim I, Goodfellow PJ: Phosphatase and tensin homologue (PTEN) pseudogene expression in endometrial cancer: a conserved regulatory mechanism important in tumorigenesis? *Gynecol Oncol* 2012, **124**:340-346.
4. Yokoyama Y, Wan X, Shinohara A, Takahashi S, Takahashi Y, Niwa K, Tamaya T: Expression of PTEN and PTEN pseudogene in endometrial carcinoma. *Int J Mol Med* 2000, **6**:47-50.
5. Zhang CL, Tada M, Kobayashi H, Nozaki M, Moriuchi T, Abe H: Detection of PTEN nonsense mutation and psiPTEN expression in central nervous system high-grade astrocytic tumors by a yeast-based stop codon assay. *Oncogene* 2000, **19**:4346-4353.
6. Hesson LB, Packham D, Pontzer E, Funchain P, Eng C, Ward RL: A reinvestigation of somatic hypermethylation at the PTEN CpG island in cancer cell lines. *Biol Proced Online* 2012, **14**:5.
7. Zysman MA, Chapman WB, Bapat B: Considerations when analyzing the methylation status of PTEN tumor suppressor gene. *Am J Pathol* 2002, **160**:795-800.
8. Hesson LB, Ward RL: Discrimination of pseudogene and parental gene DNA methylation using allelic bisulfite sequencing. *Methods Mol Biol* 2014, **1167**:265-274.

doi:10.1186/s13148-014-0033-5

Cite this article as: Hesson and Ward: The importance of distinguishing pseudogenes from parental genes. *Clinical Epigenetics* 2014 **6**:33.

\* Correspondence: l.hesson@unsw.edu.au

Adult Cancer Program, Lowy Cancer Research Centre and Prince of Wales Clinical School, UNSW, Sydney, Australia