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Short communication

Identification of a transcriptionally active *hVH-5* pseudogene on 10q22.2

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Abstract

Mitogen-activated protein kinases (MAPKs) are important regulators of a vast number of biological functions that affect life and death of eukaryotic cells and are tightly regulated by the concerted action of several phosphatases. Among these is the human homologue of *vaccinia virus* H1 phosphatase gene clone 5 (*hVH-5*) product, which dephosphorylates and thus inhibits members of the MAPK family. Here, we analyzed *hVH-5* transcripts in mammary carcinoma cell lines and discovered a sequence with 88% similarity to *hVH-5* transcripts. Because this variant of *hVH-5* lacked intronic sequences in its genomic structure, we suggest it might be a processed pseudogene of *hVH-5*. *ψhVH-5* transcripts were detected in human peripheral tissues as well as in 11 of 14 breast cancer cell lines. In respect to the normal *hVH-5* gene, the pseudogene contains several point mutations and a frame shift due to the deletion of 2 bases that would lead to the truncation of the putative *ψhVH-5* product. © 2005 Elsevier Inc. All rights reserved.

1. Introduction

Human homologue of *vaccinia virus* H1 phosphatase gene clone 5 (*hVH-5*) encodes a dually specific phosphatase that is able to dephosphorylate the regulatory phosphotyrosine as well as phosphothreonine residues of so-called mitogen-activated protein kinesis (MAPKs). MAPKs are key regulators of many cellular functions and are tightly controlled by the concerted action of different phosphatases [1,2]. Proper targeting of dual-specificity phosphatases, which are also refered to as MAPK phosphatases (MKPs), is essential for adequate signaling activity of MAPKs and thereby for several aspects of cell physiology including inflammation and cell death [3]. The phosphatase hVH-5 has a strong preference for 2 particular subclasses of MAPKs, the c-Jun N-terminal kinesis (JNKs) and p38 kinesis [4]. Both groups of MAPKs play an important role in cellular transformation, cancer progression and metastasis [3].

A potential tumor suppressor role for MKPs has been postulated [5]. Noteworthy, genes of several MKPs map to chromosomal regions whose genetic reorganizations are known to be associated with the incidence of cancer [6,7]. In particular, the chromosome region of *hVH-5* (11p15.5) has been implicated in the development of several kinds of cancer [8]. Loss of heterozygosity/allelic imbalance in breast cancer has also shown that this chromosomal location is a candidate region, which might be involved in breast cancer development [9]. These findings lead to the question whether loss or variation of *hVH-5* function and concomitantly dysregulation of MAPK signaling could be involved in tumorigenesis.

In the present work, we identified a processed pseudogene of *hVH-5,* located at 10q22.2. The *ψhVH-5* pseudogene codes for a sequence, which includes several base substitutions, insertions, and deletions compared to *hVH-5* gene sequence that would lead to a truncated protein. Transcription of the *ψhVH-5* pseudogene was detected in various human peripheral tissues as well as in 11 of 14 breast cancer cell lines.

2. Materials and methods

All cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and grown as recommended by the supplier using GIBCO cell culture reagents (Invitrogen, Karlsruhe, Germany).

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A 329 bp fragment of *hVH-5* and *hVH5*-like sequences was amplified by PCR from cDNA using following oligonucleotides: CATGGCTGGGGACCGGCTCCCG and CTCA GCAGGATGGAGAGGAAGC. PCR products were analyzed by CSGE, a non-radioactive heteroduplex based detection method for mutation screening, essentially as described by Ganguli et al. [10]. After separation, PCR products were ligated into pBluescript vectors (Stratagene, Heidelberg, Germany) and sequenced.

For the analysis of restriction fragment length polymorphisms, PCR amplificats were digested with *Taq1* and were resolved on a 12% non-denaturing polyacrylamide gel.

For the analysis of pseudogene expression, poly(A)$^+$ RNA was extracted from peripheral tissue and amplified after reverse transcription using specific primers for *ψhVH-5* (GGCTCCCGAGGAAGATGATGGAC and ATTTGGC-TGCGTGTGGCCAGCCA) and the housekeeping gene elongation factor 1a (*EF1a;* CACACGGCTCACATTGCAT and CACGAACAGCAAAGCGA).

Sequence homology search and chromosomal localization of the *ψhVH-5* pseudogene was performed by BLAST analysis of our sequence against the public human sequences from the National Center for Biotechnology Information ([11]; http://www.ncbi.nlm.gov/blast) and from the Sanger Center (http://www.ensembl.org/). For the translation prediction of sequences the computer program TRANSLATOR from JustBio (http://www.justbio.com/cgi-bin/translater/) was selected. Protein structure analysis and conserved domain search was performed using the SCANSITE algorithm ([12]; http://scansite.mit.edu).

3. Results

3.1. *Presence of a* hVH-5-*like sequence copy in breast cancer cell lines*

A 329 bp stretch from the 5′ end of the *hVH-5* coding region was reversely transcribed, amplified by RT-PCR and analyzed by CSGE. We used conformation sensitive gel electrophoresis (CSGE) to screen a total of 14 breast cancer cell lines for mutations in the amplified *hVH-5* gene fragment. As shown in Fig. 1A, 2 different *hVH-5* signals were detected. The faster migrating band in 11 of 14 cell lines indicated the presence of a transcript with *hVH-5* sequence variations. Cloning and subsequent sequencing of the 2 fragments from BT-549 cells in which both forms were similar abundant showed that we additionally found another sequence that was highly similar but clearly not identical to wild type *hVH-5* reported by Martell and colleagues [13]. Because of a G→A substitution at bp 91 (Fig. 1B), the cDNA of the *hVH-5*-like transcript lacked a *Taq1* restriction site in respect to wild type *hVH-5*. This enabled us to distinguish both transcripts by digestion of PCR amplificats as shown in Fig. 1C.

3.2. *Primary structure and chromosomal localization of* hVH-5-*like sequence*

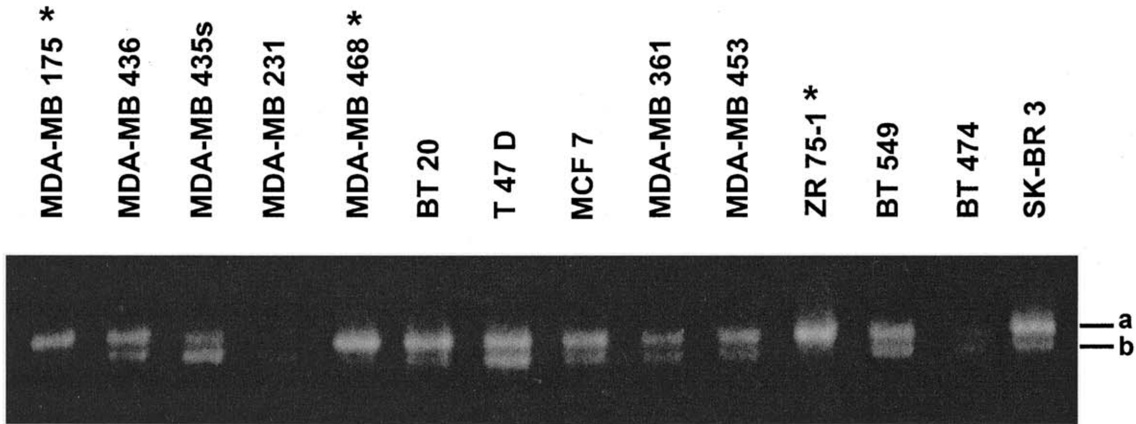
In respect to the normal *hVH-5* gene, this *hVH-5*-like sequence fragment (GeneBank accession no. AY661565) contained several nucleotide variations including a 2 base pair deletion that would lead to a frame shift and result in truncation of the protein product. As shown in Fig. 2A, this putative protein product would share only 24 of its first 27 amino acids with hVH-5 and would be terminated after 60 amino acids. Analyzing the protein sequence did not reveal any known functional domains. Performing a BLAST homology search of the human genome database we could identify a contig (GeneBank accession no. NT_008583) containing a stretch with 100% homology to the *hVH-5*-related fragment (GeneBank accession no. AY661564). The full genomic sequence of this gene nearly represents a start-to-stop-codon-length copy of the human *hVH-5* phosphatase mRNA transcript. Based on the absence of introns it can be assumed to be a processed pseudogene of *hVH-5*. The complete sequence of pseudogene *ψhVH-5* is 88% similar to *hVH-5* cDNA and contains 158-point mutations, 63 deletions, and 5 insertions in respect to the wild-type *hVH-5* sequence. We used the genome information of the NCBI database to assign the chromosomal localization of *ψhVH-5* pseudogene to chromosomal subband 10q22. The pseudogene spans an area of 1818 nucleotides and is formed of 6 exons without intervening introns. Performing the search we also detected another intronless *hVH-5*-like sequence, which mapped to 10q11.2, where another pseudogene of *hVH-5* was assumed to be located [8].

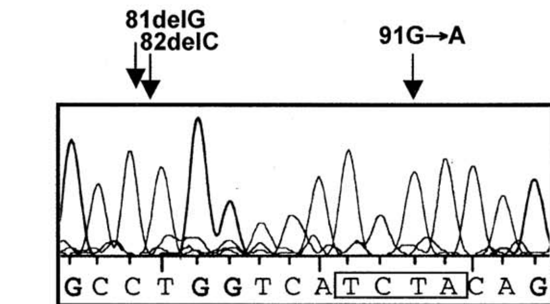
3.3. *The* ψhVH-5 *pseudogene is broadly expressed*

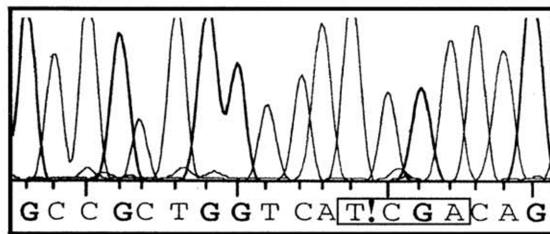
Based on sequence differences between the *hVH-5* gene and its pseudogene, *ψhVH-5*-specific primers were designed for analyzing the expression of *ψhVH-5* mRNA transcripts in different tissues by RT-PCR. To make sure that only mRNA transcripts of *ψhVH-5* were amplified, cDNA of all cell lines was checked to be free of genomic DNA (data not shown). As demonstrated in Fig. 2B, *ψhVH-5* pseudogene transcripts were readily detected in all tissues examined pointing towards a broad or even ubiquitous expression of the pseudogene.

4. Discussion

Pseudogenes are a side product of gene duplications and can arise by 2 fundamentally different processes [14,15]. First, non-processed pseudogenes are generated by gene duplication events. Second, processed pseudogenes are considered to result form reverse transcription of messenger RNA and subsequent re-integration into genomic DNA. Consequentially, this second class lacks introns and regulatory elements such as promoters and enhancers and is therefore mostly transcriptionally silent. Because the here







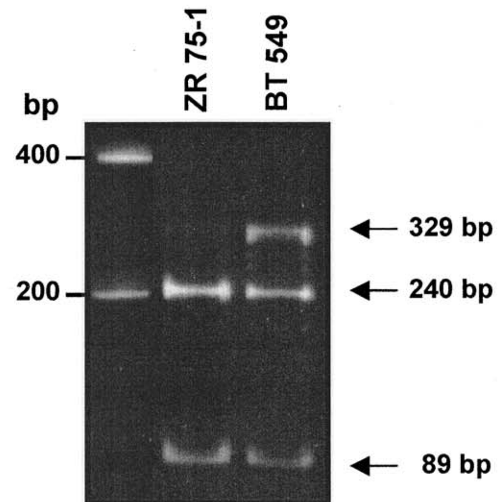
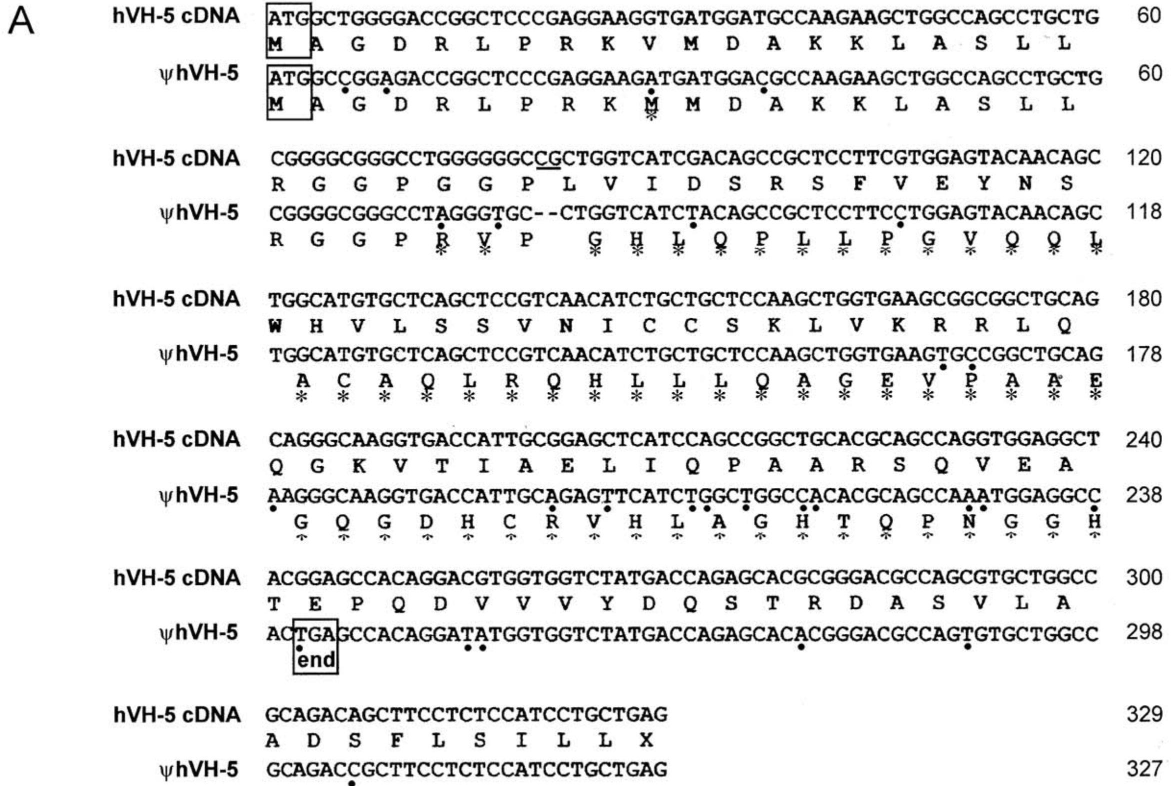


Fig. 1. Detection of a *hVH-5*-like sequence in breast cancer cell lines. *(A)* In 11 breast cancer cell lines, CSGE shows a double band that consists of a normal homoduplex *(a)* and an abnormal heteroduplex *(b)* band. A normal CSGE profile indicated by only 1 homoduplex band was detected in 3 cell lines (shown by asterisk). *(B)* Cloning and sequencing of PCR products revealed the presence of wild-type *hVH-5* and additionally an *hVH-5*-like sequence. A *Taq1* restriction enzyme site (given in a box) existing in the sequence of *hVH-5* was abolished by a G→A transition at position 91. *(C)* PCR-RFLP analysis of *hVH-5* and *hVH-5*-like amplificats from 2 cell lines.

described *ψhVH-5* pseudogene is intronless, it clearly belongs to this latter group of pseudogenes. Thus, *ψhVH-5* most likely originates from reverse transcription of *hVH-5* mRNA. In contrast to most processed pseudogenes, *ψhVH-5* was found to be transcribed in cancer cell lines as well as in normal tissue. In spite of the strikingly large number of pseudogenes estimated to be present in the human genome, only very few pseudogene transcripts have been reported so



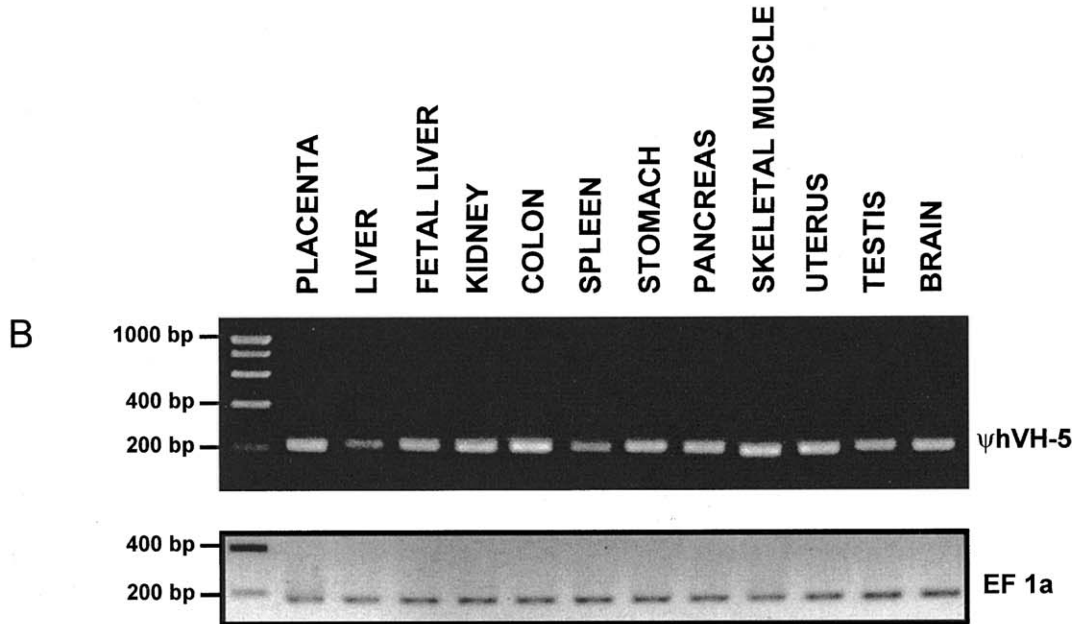


Fig. 2. Sequence and expression *ψhVH-5* pseudogene. *(A)* Partial alignment of partial DNA sequences of *ψhVH-5* and *hVH-5* and their deduced amino acid sequences. Dots and asterisks indicate alterations in DNA and amino acid sequence, respectively. Start and stop codons are boxed. *(B)* Expression of *ψhVH-5* in different tissues was analyzed by RT-PCR. In parallel transcripts of the house keeping, elongation factor 1 (EF-1), were analyzed as control.

far [16–18]. Interestingly, in the case of the tumor suppressor gene *PTEN* a pseudogene transcript was even more prevalent than the paralogous functional mRNA in some tissues and cell lines [19].

The identification of pseudogene transcripts provokes the question whether these alternative mRNAs or their protein products might have a function. The pseudogene mRNA could be translated into a protein, which retains some

function of the wild-type form. For instance, *PTEN/MMAC1* pseudogene encodes a protein that, when translated in vitro, has been shown to possess a weak but detectable dual-specificity phosphatase activity [20]. However, so far no pseudogene protein product was detected in cells [19]. In contrast, since the putative ψhVH-5 protein would be truncated and thus lack the essential phosphatase domain, it could not function as a dual specificity phosphatase. Furthermore, no domains or functional motifs have been identified in the putative protein sequence of the ψhVH-5 product. A second kind of interference could occur if the pseudogene mRNA itself would be functional. Hirotsune and colleagues challenged the popular belief that pseudogenes are simply molecular fossils by their discovery that transcripts of the pseudogene *Makorin1-p1*, are able to critically regulate the stability of the mRNA from the original *Makorin1* gene and thus gene function in development [21]. Although *Makorin1-p1* incurred many mutations during evolution that would lead to a truncated, non-functional protein, its ability to stabilize *Makorin1* mRNA was clearly dependent on the residual sequence similarity. As simililarity between the pseudogene of *hVH-5* and its functional gene is quite high with a value of 88% identity in the complete mRNA sequences (data not shown), the pseudogene we describe here might be able to interact by a similar mechanism with its functional gene.

Finally, pseudogene transcripts theoretically could contribute to mRNA degradation by RNA interference (RNAi). Transcriptionally active processed pseudogenes are devoid of the regulatory elements of the genes they arose from but are rather co-transcribed as a consequence of their proximity to promotors and enhancers of other genes. Hence, there is the possibility that pseudogenes could also be transcribed in the antisense direction and thus lead to the formation of double stranded RNA which could be cleaved by DICER into small double stranded RNAs which in turn could lead to RNA-induced silencing complex (RISC)-dependent degradation of the wild-type mRNA. The chance of such a negative interference is even higher if more than one pseudogene is present as it is the case for *hVH-5*. If the regulatory elements that lead to the transcription of the pseudogenes are active in spatial-temporal differential manner this could lead to the functional knockdown of the parental gene in particular tissues or at particular time points, for instance, in development or disease.

In conclusion, we characterized a processed pseudogene of *hVH-5* on 10q22.2. Transcription was detected in several human tissues and in 11 of 14 breast carcinoma cell lines. The putative ψhVH-5 protein would be truncated lacking any functional domain, but the RNA of *ψhVH-5* could interact with the *hVH-5* phosphatase gene by any mechanism known for non-coding RNAs.

References

[1] Saxena M, Mustelin T. Extracellular signals and scores of phosphatases: all roads lead to MAP kinase. Semin Immunol 2000;12:387–96.

[2] Keyse SM. Protein phosphatases and the regulation of mitogen-activated protein kinase signalling. Curr Opin Cell Biol 2000;12:186–92.

[3] Chang L, Karin M. Mammalian MAP kinase signalling cascades. Nature 2001;410:37–40.

[4] Johnson TR, Biggs JR, Winburn SE, Kraft AS. Regulation of dual-specificity phosphatases M3/6 and hVH-5 by phorbol esters. J Biol Chem 2000;275:31755–62.

[5] Shin DY, Ishibashi T, Choi TS, Chung E, Chung IY, Aaronson SA, Bottaro DP. A novel human ERK phosphatase regulates H-ras and v-ras signal transduction. Oncogene 1997;14:2633–9.

[6] Tanoue T, Moriguchi T, Nishida E. Molecular cloning and characterization of a novel dual specificity phosphatase, MKP-5. J Biol Chem 1999;274:19949–56.

[7] Furukawa T, Yatsuoka T, Youssef EM, Abe T, Yokoyama T, Fukushige S, Soeda E, Hoshi M, Hayashi Y, Sunamura M, Kobari M, Horii A. Genomic analysis of DUSP 6, a dual specificity MAP kinase phosphatase, in pancreatic cancer. Cytogenet Cell Genet 1998;82:156–9.

[8] Nesbit MA, Hodges MD, Campbell L, de Meulemeester TM, Alders M, Rodrigues NR, Talbot K, Theodosiou AM, Mannens MA, Nakamura S, Little PFR, Davies KE. Genomic organization and chromosomal localization of a member of the MAP kinase phosphatase gene family to human chromosome 11p15.5 and a pseudogene to 11q11.2. Genomics 1997;42:284–94.

[9] Osborne RJ, Hamshere MG. A genome-wide map showing common regions of loss of heterozygosity/allelic imbalance in breast cancer. Cancer Res 2000;60:3706–12.

[10] Ganguli A, Rock MJ, Prockop DJ. Conformation-sensitive gel electrophoresis for rapid detection of single-base differences in double-stranded PCR products and DNA fragments: evidence for solvent-induced bends in DNA heteroduplexes. Proc Natl Acad Sci USA 1993;90:10325–9.

[11] Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997;25:3389–402.

[12] Yaffe MB, Leparc GG, Lai J, Obata T, Volinia S, Cantley LC. A motif-based profile scanning approach for genome wide prediction of signaling pathways. Nature Biotech 2001;19:348–53.

[13] Martell KJ, Seasholtz AF, Kwak SP, Clemens KK, Dixon JE. hVH-5: A protein tyrosine phosphatase abundant in brain that inactivates mitogen-activated protein kinase. J Neurochem 1995;65:1823–33.

[14] Harrison PM, Hegyi H, Balasubramanian S, Luscombe NM, Bertone P, Echols N, Johnson T, Gerstein M. Molecular fossils in the human genome: identification and analysis of the pseudogenes in chromosomes 21 and 22. Gen Res 2002;12:272–80.

[15] Vanin EF. Processed pseudogenes: characteristics and evolution. Annu Rev Genet 1985;19:253–72.

[16] Soares MB, Schon E, Henderson A, Karathanasis SK, Kate R, Zeitlin S, Chirgwin J, Efstratiadis A. RNA-mediated gene duplication: the rat preproinsulin I gene is a functional retroposon. Mol Cell Biol 1985;5:2090–103.

[17] Bristow J, Gitelman SE, Tee MK, Staels B, Miller WL. Abundant adrenal-specific transcription of the human P450c21A "pseudogene". J Biol Chem 1993;268:12919–24.

[18] Olsen MA, Schechter LE. Cloning, mRNA localization and evolutionary conservation of a human 5-HT7 receptor pseudogene. Gene 1999;227:63–9.

[19] Fuji GH, Morimoto AM, Berson AE, Bolen JB. Transcriptional analysis of the PTEN/MMAC1 pseudogene, ψPTEN. Oncogene 1999;18:1765–9.

[20] Myers MP, Stolarov JP, Eng C, Li J, Wang SI, Wigler MH, Parsons R, Tonks NK. P-TEN, the tumor suppressor from human chromosome 10q23, is a dual specificity phosphatase. Proc Natl Acad Sci USA 1997;94:9052–7.

[21] Hirotsune S, Yoshida N, Chen A, Garrett L, Sugiyama F, Takahashi S, Yagami K, Wynshaw-Boris A, Yoshiki A. An expressed pseudogene regulates the messenger-RNA stability of its homologous coding gene. Nature 2003;423:91–6.