Correspondence: Daniel Sinnett, Ph.D., Division of Hematology-Oncology, Sainte-Justine Hospital Research Center, 3175 Chemin de la Côte-Sainte-Catherine, Montreal, H3T 1C5, Canada. Phone: international +514.3454931 # 2990. Fax: international +514.3454731. E-mail: daniel.sinnett@umontreal.ca

Citation: Healy J, Roy-Gagnon M-H, Sinnett D. No evidence for association between TGFB1 promoter SNPs and the risk of childhood pre-B acute lymphoblastic leukemia among French Canadians. Haematologica 2009;94:1034-1036. doi: 10.3324/haematol.2009.005991

References

1. Elliott RL, Blobe GC. Role of transforming growth factor β in human cancer. J Clin Oncol 2005;23:2078-93.
2. Dunning AM, Ellis PD, McBride S, Kirschenlohr HL, Healey CS, Kemp PR, et al. A transforming growth factor β1 signal peptide variant increases secretion in vitro and is associated with increased incidence of invasive breast cancer. Cancer Res 2003;63:2610-5.
3. Kang HG, Chae MH, Park JM, Kim EJ, Park JH, Kam S, et al. Polymorphisms in TGF-β1 gene and the risk of lung cancer. Lung Cancer 2006;52:1-7.
4. Saltzman BS, Yamamoto JF, Decker R, Yokochi L, Theriault AG, Vogt TM, et al. Association of genetic variation in the transforming growth factor β-1 gene with serum levels and risk of colorectal neoplasia. Cancer Res 2008;68:1236-44.
5. Ewart-Toland A, Chan JM, Yuan J, Balmain A, Ma J. A gain of function TGFB1 polymorphism may be associated with late stage prostate cancer. Cancer Epidemiol Biomarkers Prev 2004;13:759-64.
6. Healy J, Belanger H, Beaulieu P, Lariviere M, Labuda D, Sinnett D. Promoter SNPs in G1/S checkpoint regulators and their impact on the susceptibility to childhood leukemia. Blood 2007;109:683-92.
7. Sinnett D, Beaulieu P, Belanger H, Lefebvre JF, Langlois S, Theberge MC, et al. Detection and characterization of DNA variants in the promoter regions of hundreds of human disease candidate genes. Genomics 2006;87:704-10.
8. Labuda D, Krajinovic M, Richer C, Skoll A, Sinnett H, Yotova V, et al. Rapid detection of CYP1A1, CYP2D6, and NAT variants by multiplex polymerase chain reaction and allele-specific oligonucleotide assay. Anal Biochem 1999;275:84-92.
9. O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet 1998;63:259-66.
10. Becker T, Knapp M. Maximum-likelihood estimation of haplotype frequencies in nuclear families. Genet Epidemiol 2004;27:21-32.
11. Becker T, Knapp M. A powerful strategy to account for multiple testing in the context of haplotype analysis. Am J Hum Genet 2004;75:561-70.
12. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J R Stat Soc 1995;57:289-300.

Physiological *PTEN* expression in peripheral T-cell lymphoma not otherwise specified

Peripheral T-cell lymphomas not otherwise specified (PTCL/NOS) is the commonest subtype of PTCL.[1] This is a complex entity, characterized by great morphologic, immunophenotypic and clinical variability, whose molecular pathology is still largely unknown.[2]

Recently, gene expression profiling (GEP) studies allowed the identification of PTCL/NOS-associated signatures, leading to the better understanding of its histogenesis, pathogenesis and prognostication.[2] Interestingly, proliferation pathways were found to be commonly altered in PTCL,[2,3] highly proliferative cases being characterized by poorer prognosis.[4,5]

The tumor suppressor PTEN (phosphatase tensin homolog), is a critical regulator for multiple cellular processes including proliferation. In addition to its well-defined role in signaling in the cytoplasm, its nuclear localization seems to contribute to its physiological tumor suppressor activity. Interestingly, somatic mutations of PTEN, including null or missense mutations, and truncations, occur in multiple sporadic tumors. Furthermore, deletions of 10q22-25 have been reported in ~5-10% of B-NHLs, raising the possibility of PTEN involvement in the pathogenesis of lymphoid malignancies.[6] Importantly, the great majority of such mutations abolish (81%) or greatly decrease (10%) PTEN phosphatase activity, by determining the absence of the encoded product or the presence of a mutated, non-functional protein, finally leading to tumorigenesis.

Interestingly, experimental evidence showed that mice with loss of heterozygosity of the wildtype *mPTEN* allele develop T-cell lymphomas.[7] Furthermore, down-regulation of this molecule was found to be essential for the formation of the typical nuclear lobules in adult T-cell leukemia/lymphoma[8] and its alterations may be important in the pathogenesis and progression of mycosis fungoides[9] and T-cell acute lymphoid leukemia.[10] Notably, partial or complete loss of PTEN was detected in 66.7% of anaplastic large cell lymphomas and in 12.5% of the few other mature T-/NK-cell lymphomas so far studied.[11] Nevertheless, the number of PTCLs/NOS was indeed limited and no definitive conclusion can be drawn concerning the possible involvement of PTEN in the pathogenesis of such tumors. The present study was designed to evaluate the possible occurrence of PTEN aberration in PTCL/NOS. We analyzed GEP data of 28 PTCLs/NOS, and 20 samples of normal T cells. Technical details have been previously reported (*http://www.ncbi.nlm.nih.gov/projects/geo/*).[3,12]

Genotypes were determined by PCR amplification and direct sequencing. In particular, PCR products of all nine exons and exon-intron junctions of PTEN were directly sequenced in 72 PTCLs/NOS. Primers and relative conditions of amplification are detailed in Table 1.

Finally, we studied PTEN expression by immunohistochemistry (IHC) on tissue micro-arrays (TMAs) containing 34 PTCL/NOS cases.[4] PTEN cytoplasmatic expression was tested by a mouse monoclonal antibody, PTEN/MMAC1 Ab-4 (clone 17.A, Thermo Scientific), at a 1:20 dilution, while PTEN nuclear expression was tested by a specific mouse monoclonal antibody, PTEN Ab-6 (clone 28H6, Thermo Scientific), which was applied at a 1:10 dilution. The sections underwent antigen retrieval in citrate buffer (pH=6.0) in a micro-waver at 900W (3 cycles lasting 5' each) and revealed by the EnVision and APAAP techniques, respectively.

First, GEP showed no significant differences in *PTEN* expression when PTCL/NOS cases were compared to normal T-lymphocytes (Figure 1).

Secondly, direct sequencing did not reveal any significant abnormality. Similarly, PTEN locus was found to be intact in the course of a high-density karyotyping study carried out by the Affymetrix 250k SNPs-array in a series of PTCL/NOS (*Hartmann et al., manuscript in preparation*).

We then studied the protein expression and localization in a series of 34 PTCL/NOS cases. Importantly, IHC documented physiological nuclear and cytoplasmic expression in 31/31 and 23/26 evaluable cases, respec-

Table 1. Primers for *PTEN* PCR analysis and sequencing, and relative annealing temperatures.

Exon	Forward primer (5' to 3')	Reverse primer (5' to 3')	PCR annealing temperature (°C) (t.a.)
1	CAAGTCCAGAGCCATTTCCAT	AAGAGGAGCAGCCGCAGAAAT	56
2	ATTCACATGTAACTTCTTTTA	CAACATGAATATAAACATCAA	56
3	TAATTTCAAATGTTAGCTCAT	AAGATATTTGCAAGCATACAA	56
4	GTTTGTTAGTATTAGTACTTT	ACAACATAGTACAGTACATTC	56
5	CTAAAGTTACCTACTTGTTAAT	AGGAAAAACATCAAAAAATAAC	54
6	ATATGTTCTTAAATGGCTACG	ACATGGAAGGATGAGAATTTC	55
7	CGTGTATATTGCTGATATTAAT	CTCCCAATGAAAGTAAAGTACA	55
8	ATGTTTAACATAGGTGACAGA	ACACATCACATACATACAAGT	55
9	GTTTAAGATGAGTCATATTTG	TGGTGTTTTATCCCTCTTGAT	55

PCR conditions were: 35 cycles of denaturation (95°C for 60 sec), an annealing step (t.a. for 60 sec), an extension step (72°C for 60 sec), finally 7 min of extension at 72°C. For sequencing we used the same reverse primers of PCR reaction.

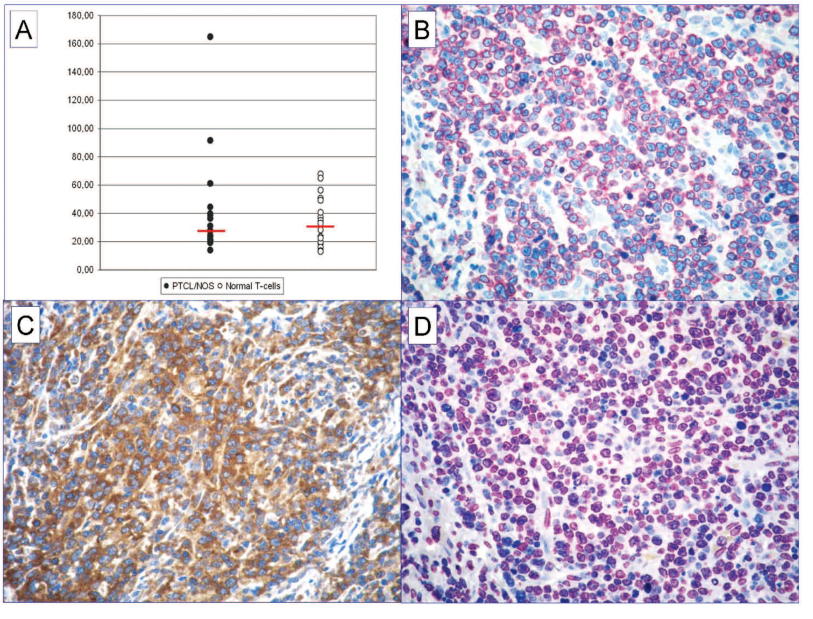

Figure 1. (A) Normalized expression values of *PTEN* in PTCL/NOS cases *vs.* samples of normal tissues evaluated by gene expression analysis on DNA microarrays. Median values are indicated by bars. No significant differences in *PTEN* expression was observed in PTCL/NOS cases compared to normal tissues. (B) CD3 immunostaining in a PTCL/NOS sample (APAAP Technique; x400). Note the high amount of CD3+ neoplastic elements. (C) Cytoplasmatic PTEN immunostaining in a PTCL/NOS sample (Immunoperoxidase Envision+ Technique; Gill's hematoxylin counterstaining; Olympus BX41 microscope, Olympus CAMEDIA C-7070 camera, magnification x400, colors corrected after acquisition with Adobe Photoshop). (D) Nuclear PTEN immunostaining in a PTCL/NOS sample (APAAP Technique; Olympus BX41 microscope, Olympus CAMEDIA C-7070 camera, magnification x400, colors corrected after acquisition with Adobe Photoshop).

tively (Figure 1). Notably, the 3 cases with nuclear positivity alone lacked genomic abnormalities: they were then considered to be normal as far as PTEN expression is concerned. Possibly, in these cases, the cytoplasmatic negativity reflected a defective immunohistochemical reaction.

In a previous study, PTEN loss was found by IHC in only 3/24 mature T/NK lymphomas, suggesting the possibility of occasional PTEN involvement in PTCL/NOS pathogenesis. However, the number of PTCL/NOS with such a pattern was not specified, genomic analyses were not performed, and possible IHC reaction failure was not discussed. Thus, based on the present data, obtained in the largest series of PTCLs/NOS so far studied with this purpose, we can conclude that PTEN seems to have physiological distribution in PTCL/NOS. In fact, PTEN was transcriptionally regulated in tumors as in normal T-lymphocytes, lacked structural abnormalities and presented with regular expression in both cytoplasmatic and nuclear compartments. Anyway, it would be of interest to study matched PTCL/NOS cases collected at diagnosis and relapse, respectively, in order to evaluate a possible involvement of PTEN in PTCL/NOS progression.

Anna Gazzola, Clara Bertuzzi, Claudio Agostinelli, Simona Righi, Stefano A. Pileri, and Pier Paolo Piccaluga**

** These authors equally contributed*

Department of Hematology and Oncology "L. and A. Seràgnoli", Hematopathology Section, S. Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy

Key words: peripheral T-cell lymphoma, PTEN, microarray, immunohistochemistry, gene sequencing.

Acknowledgments: the authors are grateful to Dr. Maria Teresa Sista for tissue micro-array construction and to Dr. Maria Rosaria Sapienza for gene sequencing.

Funding: this work was supported by BolognAIL, Ateneo 60% (Prof. S. A. Pileri), Centro Interdipartimentale per la Ricerca sul Cancro "G. Prodi", AIRC, Fondazione CARISBO, Progetto Strategico d'Ateneo (Prof. SA Pileri/Dott. PP Piccaluga) grants.

Correspondence: Pier Paolo Piccaluga, MD, PhD, Molecular Pathology Laboratory, Section of Hematopathology, Department of Hematology and Oncology "L. and A. Seràgnoli", S. Orsola-

Malpighi Hospital, University of Bologna, Via Massarenti, 9 40138 Bologna, Italy. Phone: international +39.051.6364043. Fax: international +39.051.6364037. E-mail: pierpaolo.piccaluga@unibo.it

Citation: Gazzola A, Bertuzzi C, Agostinelli C, Righi S, Pileri SA, Piccaluga PP. Physiological PTEN expression in peripheral T-cell lymphoma not otherwise specified. Haematologica 2009; 94:1036-1038. doi: 10.3324/haematol.2009.006718

References

1. Swerdlow S, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. Editors. WHO Classification of tumors of hematopoietic and lymphoid tissues. IV ed. Lyon; IARC. 2008.
2. Agostinelli C, Piccaluga PP, Went P, Rossi M, Gazzola A, Righi S, et al. Peripheral T cell lymphoma, not otherwise specified: the stuff of genes, dreams and therapies. J Clin Pathol 2008;61:1160-7.
3. Piccaluga PP, Agostinelli C, Califano A, Rossi M, Basso K, Zupo S, et al. Gene expression analysis of peripheral T cell lymphoma, unspecified, reveals distinct profiles and new potential therapeutic targets. J Clin Invest 2007;117: 823-34.
4. Went P, Agostinelli C, Gallamini A, Piccaluga PP, Ascani S, Sabattini E, et al. Marker expression in peripheral T-cell lymphoma: a proposed clinical-pathologic prognostic score. J Clin Oncol 2006;24:2472-9.
5. Cuadros M, Dave SS, Jaffe ES, Honrado E, Milne R, Alves J, et al. Identification of a proliferation signature related to survival in nodal peripheral T-cell lymphomas. J Clin Oncol 2007;25:3321-9.
6. Butler MP, Wang SI, Chaganti RS, Parsons R, Dalla-Favera R. Analysis of PTEN mutations and deletions in B-cell non-Hodgkin's lymphomas. Genes Chromosomes Cancer 1999;24:322-7.
7. Suzuki A, de la Pompa JL, Stambolic V, Elia AJ, Sasaki T, del Barco Barrantes I, et al. High cancer susceptibility and embryonic lethality associated with mutation of the PTEN tumor suppressor gene in mice. Curr Biol 1998; 8:1169-78.
8. Fukuda R, Hayashi A, Utsunomiya A, Nukada Y, Fukui R, Itoh K, et al. Alteration of phosphatidylinositol 3-kinase cascade in the multilobulated nuclear formation of adult T cell leukemia/lymphoma (ATLL). Proc Natl Acad Sci USA 2005;102:15213-8.
9. Scarisbrick JJ, Woolford AJ, Russell-Jones R, Whittaker SJ. Loss of heterozygosity on 10q and microsatellite instability in advanced stages of primary cutaneous T-cell lymphoma and possible association with homozygous deletion of PTEN. Blood 2000;95:2937-42.
10. De Keersmaecker K, Marynen P, Cools J. Genetic insights in the pathogenesis of T-cell acute lymphoblastic leukemia. Haematologica 2005;90:1116-27.
11. Uner AH, Saglam A, Han U, Hayran M, Sungur A, Ruacan S. PTEN and p27 expression in mature T-cell and NK-cell neoplasms. Leuk Lymphoma 2005;46:1463-70.
12. Piccaluga PP, Agostinelli C, Righi S, Zinzani PL, Pileri SA. Expression of CD52 in peripheral T-cell lymphoma. Haematologica 2007;92:566-7.