WTX is rarely mutated in acute myeloid leukemia

WTX (Wilms' tumor gene on the X chromosome) is inactivated in 30% of Wilms' tumors, mostly by chromosomal deletion.[1] The *WTX* protein is a component of the β-catenin destruction complex and promotes its ubiquitination and degradation, functioning as a negative regulator of WNT/β-catenin signaling.[2] Several recent studies support a role for β-catenin in the pathogenesis of acute myeloid leukemia (AML), including evidence that β-catenin phosphorylation is regulated by FLT3,[3] that β-catenin overexpression is associated with poor prognosis in AML,[4] and that several components of the β-catenin complex are subject to rare mutation in AML.[5]

Rivera and co-workers noted that *WTX* mutations in Wilms' tumors occurred independent of mutations in Wilms' tumor 1 (*WT1*),[1] the first gene linked to Wilms' tumor and accounting for 10-20% of cases.[6] Our group recently confirmed that *WT1* is mutated in 11% of normal karyotype (NK) AML[7] and we therefore looked for the presence of aberrations in the *WTX* gene in NK AML.

The role of *WTX* in AML was explored using a combination of PCR-based mutation screening, fluorescence *in situ* hybridization (FISH) and gene expression analyses. Cryopreserved leucopheresis or bone marrow samples from 60 patients with newly diagnosed NK AML were selected for investigation after obtaining informed consent, in accordance with the Declaration of Helsinki. *WTX* mutation analysis was performed on genomic DNA from all patients (33 female, 27 male; median age at diagnosis 55 years). The molecular features of the samples were representative of *de novo* NK AML with 65% with *NPM1* mutations and 38% with *FLT3* internal-tandem duplications. PCR-based sequencing analysis of the *WTX* gene was restricted to a 1.5 Kb mutational hotspot within *WTX* in which all mutations were reported in the Wilms' tumor cases. Details of the PCR conditions and primers are available on request. FISH was performed according to standard methods using fixed cells in 27 patient samples (16 female, 11 male; median age at diagnosis 52 years) in which sufficient stored cells were available for analysis. The cells were analyzed by dual color interphase FISH using the probes previously selected by Rivera and co-workers, produced from BAC clones RP11-1105p13 and RP11-54p15 (BACPAC Resources Center, Oakland, CA, USA). The RP11-1105p13 clone is located at the 5' untranslated region of *WTX* while the control clone, RP11-54p15 locates to a region 5Mb telomeric to *WTX*. Gene expression analysis was performed using RNA extracted from 40 distinct NK AML samples (no overlap with the 60 samples used for mutation analysis and FISH). The Human Genome U133A Plus 2.0 Gene Chip (Affymetrix, Santa Clara, CA, USA), which contains 54, 675 ProbeSets, was used following the manufacturer's protocols. The raw data was imported into Partek software and normalized using RMA.

A single putative mutation was detected among the 60 analyzed NK AML cases. The missense mutation arose in an evolutionarily conserved residue ($Lys^{292}\rightarrow Asn^{292}$) (Figure 1A) and is identical to a mutation reported by Rivera *et al*. in their Wilms' tumor series. Although a remission sample was not available for comparison to exclude the possibility of a rare polymorphism, $Lys^{292}\rightarrow Asn^{292}$ was confirmed as an acquired mutation in the Wilms' tumor case by comparison with non-tumor tissue from the same individual.[1] A previously described non-synonymous single nucleotide polymorphism (SNP) was detected in 2 patient samples ($Phe^{159}\rightarrow Leu^{159}$) and a silent SNP at amino acid 145 was noted in a single sample. Deletion was more common than mutation in Wilms' tumor (22% *vs.* 8%);[1] however, we excluded deletions in all 27 AML samples examined by FISH (Figure 1B). These data were also consistent with PCR amplification results in male samples in which all patient DNAs amplified satisfactorily. It should be noted, however, that this approach has potential limitations. The FISH strategy used here is not appropriate for detecting small intragenic deletions and the restricted sequencing would not detect aberrations, which are located outside the mutation hotspot region of *WTX*.

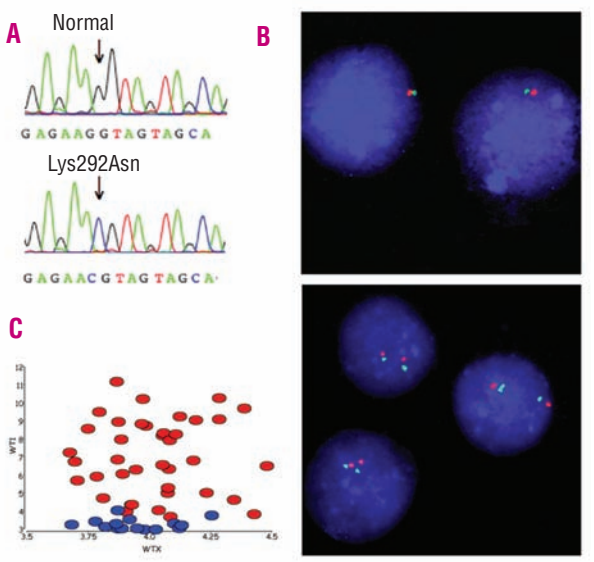

Figure 1. A. The genomic DNA sequence tracings for a normal sequence (upper panel) and the putative missense mutation (lower panel). Arrows indicate the position of the single nucleotide substitution, a C for G at position 876, leading to Lys292Asn. **B.** Interphase FISH analysis of 2 AML samples demonstrating no gross deletion within the *WTX* gene. One set of probes is noted within each cell in the upper photo (male) and 2 sets of probes in the lower photo (female). Probes for the 5' untranslated region of the *WTX* gene (RP11-1105p13) are labeled in green and probes covering a region 5MB telomeric to the *WTX* gene (RP11-54p15) are labeled in red. **C.** A comparison of *WT1* and *WTX* gene expression ratios in AML (red) and normal bone marrow (blue) cells, using a gene expression array. Each circle represents 1 AML or normal bone marrow patient sample.

Since *WT1* is overexpressed in 80% of AML cases,[8] the expression of *WTX* in AML cells was examined to determine if it was altered in comparison to normal hematopoiesis (Figure 1C). *WT1* showed higher expression in AML compared with 15 normal bone marrow samples. By contrast, expression of *WTX* in AML samples was not significantly different from its expression in normal bone marrow cells. These data differ from studies in normal kidney where Rivera and co-workers noted that the pattern of *WT1* and *WTX* expression, when examined over time in the kidney, was virtually identical. Both genes were expressed in the condensing

metanephric mesenchyme and in early epithelial structures that are the precursors to glomeruli and are the presumed precursors of Wilms' tumors.[1] The similar temporal patterns of *WT1* and *WTX* expression in the kidney suggest that these proteins may have similar roles in kidney development and may lead to tumor formation via similar pathways.

The role of WT1 in normal and malignant hematopoiesis has still not been clearly defined while WTX's role has not yet been examined. WT1 has been implicated as a cell cycle regulator promoting proliferation, apoptosis and differentiation, and is thought to act in different situations, both as a tumor suppressor and as an oncogene.[9] Functional studies of *WTX* suggest that it acts as a tumor suppressor gene in renal cells by negatively regulating WNT/β-catenin signaling.[2] Aberrant activation of WNT/β-catenin signaling has been associated with several cancers.[10] *WTX* 's association with the WNT pathway is also consistent with previous reports of constitutive activation of the WNT/β-catenin signaling pathway by mutations in the β-catenin gene in Wilms' tumor.[11]

Murine models have demonstrated that WNT signaling is also involved in hematopoietic progenitor cell proliferation and self-renewal.[12] In contrast to Wilms' tumor, β-catenin mutations have not been observed in AML but constitutive activation and overexpression of β-catenin occurs[13] and is associated with a poorer survival in AML patients.[4] The association between *WT1* and *WTX* expression in renal cells and the association between *WT1* and β-catenin mutations in Wilms' tumor support the hypothesis that *WT1* and *WTX* may be involved in similar cellular pathways in kidney development. However, mutations in *WT1* and *WTX* were mutually exclusive in Wilms' tumor, suggesting that the two proteins may function by separate pathways to achieve a similar neoplastic outcome.[1] Therefore, WTX may exert its neoplastic effect via the WNT/β-catenin pathway in Wilms' tumor but according to this current data, it is unlikely to explain aberrant WNT signaling activation in AML.

Carolyn Owen,[1] Priya Virappane,[1] Mary Alikian,[1] Irina Stasevich,[1] Karin Summers,[1] Debbie Lillington,[1] Dominique Bonnet,[2] Alan Burnett,[3] Ken Mills,[4] T. Andrew Lister,[1] and Jude Fitzgibbon[1]

[1]Centre for Medical Oncology, Barts and the London School of Medicine & Dentistry, London; [2]Cancer Research UK, London Research Institute, London; [3]Department of Haematology, School of Medicine, Cardiff University, Cardiff; [4]Centre for Cancer Research and Cell Biology, Queens University Belfast, Belfast, UK

Key words: AML, WTX, mutation.

Correspondence: Carolyn Owen, MD, FRCPC, Centre for Medical Oncology, Institute of Cancer, Queen Mary School of Medicine & Dentistry, Charterhouse Square, London EC1M 6BQ, UK. Phone: international +44.207.8825616. Fax: international +44.2078826004. E-mail: carolyn.owen@cancer.org.uk

References

1. Rivera MN, Kim WJ, Wells J, Driscoll DR, Brannigan BW, Han M, et al. An X chromosome gene, WTX, is commonly inactivated in Wilms tumor. Science 2007;315:642-5.
2. Major MB, Camp ND, Berndt JD, Yi X, Goldenberg SJ, Hubbert C, et al. Wilms tumor suppressor WTX negatively regulates WNT/β-catenin signaling. Science 2007;316:1043-6.
3. Kajiguchi T, Chung EJ, Lee S, Stine A, Kiyoi H, Naoe T, et al. FLT3 regulates β-catenin tyrosine phosphorylation, nuclear localization, and transcriptional activity in acute myeloid leukemia cells. Leukemia 2007;21:2476-84.
4. Ysebaert L, Chicanne G, Demur C, De Toni F, Prade-Houdellier N, Ruidavets JB, et al. Expression of β-catenin by acute myeloid leukemia cells predicts enhanced clonogenic capacities and poor prognosis. Leukemia 2006;20:1211-6.
5. Lilleberg SL, Hempel J, Durocher J, Foster D, Hartman C, Stoddard J, et al. High-throughput genetic profiling of acute myeloid leukemias: novel mutations detected in signal transduction pathway genes by a strategy utilizing endonuclease-based heteroduplex scanning and DNA sequencing. ASH Annual Meeting Abstracts 2006;108:1447a[Abstract].
6. Call KM, Glaser T, Ito CY, Buckler AJ, Pelletier J, Haber DA, et al. Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus. Cell 1990;60:509-20.
7. Summers K, Stevens J, Kakkas I, Smith M, Smith LL, Macdougall F, et al. Wilms' tumour 1 mutations are associated with FLT3-ITD and failure of standard induction chemotherapy in patients with normal karyotype AML. Leukemia 2007;21:550-1.
8. Inoue K, Sugiyama H, Ogawa H, Nakagawa M, Yamagami T, Miwa H, et al. WT1 as a new prognostic factor and a new marker for the detection of minimal residual disease in acute leukemia. Blood 1994;84:3071-9.
9. Ariyaratana S, Loeb DM. The role of the Wilms tumour gene (WT1) in normal and malignant haematopoiesis. Expert Rev Mol Med 2007;9:1-17.
10. Reya T, Clevers H. Wnt signalling in stem cells and cancer. Nature 2005;434:843-50.
11. Koesters R, Ridder R, Kopp-Schneider A, Betts D, Adams V, Niggli F, et al. Mutational activation of the β-catenin proto-oncogene is a common event in the development of Wilms' tumors. Cancer Res 1999;59:3880-2.
12. Austin TW, Solar GP, Ziegler FC, Liem L, Matthews W. A role for the Wnt gene family in hematopoiesis: expansion of multilineage progenitor cells. Blood 1997;89:3624-35.
13. Simon M, Grandage VL, Linch DC, Khwaja A. Constitutive activation of the Wnt/β-catenin signalling pathway in acute myeloid leukaemia. Oncogene 2005;24:2410-20.

Citation: Owen C, Virappane P, Alikian M, Stasevich I, Summers K, Lillington D, Bonnet D, Burnett A, Mills K, Lister TA, Fitzgibbon J. WTX is rarely mutated in acute myeloid leukemia. Haematologica 2008 June; 93(6):947-948. doi: 10.3324/haematol.12509