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leukocytes (mostly granulocytes), the clinical presence of some degree of (hepato)splenomegaly, and overt bleeding tendency combined with a simple screening test for neutrophils.

Although all beta-integrins are normally expressed, the activation mechanism of beta-2 and beta-3 integrins is disturbed, with Rap1 a clear candidate for causative mutations. Whereas Rap1a is ubiquitously expressed, Rap1b is expressed in hematopoietic cells in particular. The reduced aggregation response in *Rap1b*$^{-/-}$ mice was overcome at higher doses of collagen, which differs from the responses observed in the patients described, making us doubt whether Rap1 activation was really involved. This was subsequently shown by our in vitro experiments in a number of patients, with a normal Rap1 activation in neutrophils.[2]

On the other hand, Alon et al initially described an Arab patient with defective Rap1 activation in an Epstein-Barr virus (EBV)–B-cell line upon stimulation with chemokines but with normal Rap1 activation when directly activated by phorbol ester.[3] In 2 other patients the authors have now detected splice site mutations in *CalDAG-GEF1*.[4] Although not determined, Rap1 activation is presumed to be again disturbed, in contrast to our own observations. Etzioni and Alon also refer to the paper of Crittenden et al, who showed platelet aggregation defects, and that of Bergmeier et al, who are now describing abnormal leukocyte mobilization in experimental inflammation models in *CalDag-Gef1* knockout mice.[5,6] Thus, some of the patients with a clinical syndrome of a combined leukocyte and platelet adhesion defect may suffer from abnormalities in CalDAG-GEF1 regulation.

In our Turkish families, there are some (partly unpublished) results that are clearly different. First, we have found that none of the LAD-1/variant patients showed defective Rap1 activation.[2]

Second, using both monoclonal antibodies as well as polyclonal antibodies (in fact, the same as used by Etzioni and Alon), we have detected normal levels of the CalDAG-GEF-1 protein with the expected size and no reduced or variant proteins of a smaller molecular weight in the lysates of EBV cell lines or neutrophils from the patients. This is in contrast with results obtained in the 2 patients described by Etzioni and Alon.

Third, we tested *CalDAG-GEF1* as an obvious candidate gene in our patients about a year ago but could not detect any mutation in the gene encoding this key enzyme in Rap1 activation at the genomic or the cDNA level.

Finally, we have localized the defect in a different gene unrelated to Rap1 metabolism. In all our families there is a homozygous deletion currently under investigation. The small deletion we found in homozygous form in the patients and in heterozygous form in the parents was not present in more than 80 unrelated Turkish individuals tested thus far.

Together, this fits with the idea that the LAD-1/variant syndrome does not cluster with LAD-III.

Even though an update has been published on patient groupings (ESID Meeting, Budapest, Hungary, October 5-8, 2005), including the name "LAD-III" for all patients with beta-integrin activation defects, this is merely a reflection of the urge to make life easier for medical practice and clinical immunologists. At this moment, we believe that premature clustering of all these syndromes into one category would be a mistake, very much like the same misleading grouping nowadays recognized in another clinical defect known as common variable immunodeficiency (CVID).[7]

Therefore, we will refrain from the suggestion by Etzioni and Alon to cluster all beta-integrin activation defects until we fully understand the defect of our patients.

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To the editor:

WNT5A is epigenetically silenced in hematologic malignancies and inhibits leukemia cell growth as a tumor suppressor

Aberrant activation of the Wnt/β-catenin signaling pathway is a hallmark of cancers.[1,2] A recent *Blood* report showed the down-regulation of multiple Wnt-signaling inhibitors including *SFRPs, WIF1, DKK3,* and *DACT1* in acute lymphoblastic leukemia (ALL) cell lines and tumors due to promoter methylation, leading to the activation of Wnt signaling.[3]

WNT5A is another noncanonical and nontransforming Wnt protein.[4] Although there are conflicting data regarding its expression and functions in tumorigenesis, WNT5A signals through the noncanonical Wnt/Ca^{++} pathway to block the Wnt-signaling cascade and inhibits B-cell proliferation.[5] Its expression was down-regulated in most acute leukemia patients.[5]

We identified *WNT5A* as an up-regulated gene in nasal NK/T-cell lymphoma (NL) after 5-aza-2′-deoxycitidine (Aza) global demethylation and microarray expression analysis (Figure 1A), indicating that *WNT5A* is a methylation-silenced gene. Semiquantitative reverse transcriptase–polymerase chain reaction (RT-PCR) showed its down-regulation or silencing in multiple hematologic

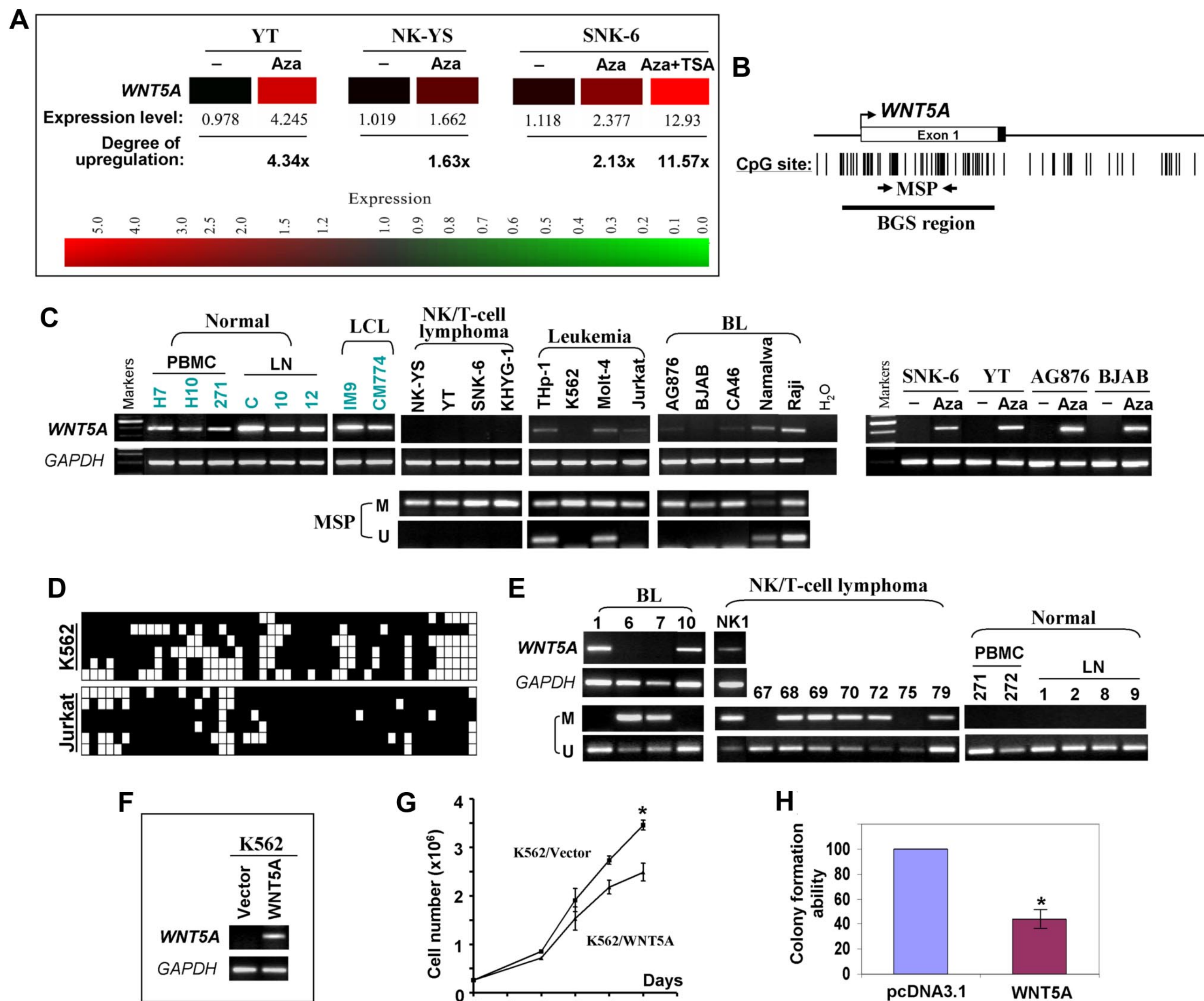

Figure 1. WNT5A is epigenetically silenced in hematologic malignancies and inhibits leukemic cell growth. (A) Expression levels of *WNT5A* mRNA in 5-aza-2′-deoxycytidine (Aza)–treated and untreated nasal NK/T-cell lymphoma (NK-YS and SNK-6) and NK leukemia (YT) cell lines. The data were obtained with microarray (Human Genome U133 Plus 2.0 Microarray, Affymetrix, Santa Clara, CA) expression analysis by GeneSpring version 7.3.1 software (Agilent, Palo Alto, CA). The degree of *WNT5A* up-regulation after Aza and TSA treatment is also shown. Red color represents high expression whereas green color represents low expression. (B) The CpG island (CGI) of the *WNT5A* promoter includes the core promoter, exon 1, and part of intron 1. The transcription start site is indicated by a curved arrow. The MSP and BGS regions analyzed in the CGI are indicated. (C) Semiquantitative RT-PCR and MSP analyses of *WNT5A* in normal cells and tissues and tumor cell lines. *WNT5A* expression in cell lines after treatment with 5 μM Aza[7] is shown on the right. *GAPDH* was used as a control. The *WNT5A* expression levels among different PBMC samples could be variable. M indicates methylated; and U, unmethylated. (D) High-resolution methylation analysis of the *WNT5A* promoter in 2 leukemia cell lines by BGS, showing the methylation status of every CpG site in the studied region. Each row in the grid represents an individual allele of the *WNT5A* promoter. ■ and □ represent methylated or unmethylated CpG sites, respectively. (E) Representative MSP results of primary BL, nasal NK/T-cell lymphoma, normal PBMCs, and LN tissues. RT-PCR results of several primary BL and 1 nasal NK/T-cell lymphoma are also shown. (F) Expression levels of *WNT5A* in silenced K562 cells before and after transfection were determined by RT-PCR. (G) Growth curves of K562 cells after transfection with pcDNA3.1-WNT5A or control vector. At indicated time points after transfection, cell numbers were counted and plotted. Mean values plus or minus SD of triplicate experiments are shown (*$P < .05$). (H) Quantitative analysis of colony numbers after transfection with pcDNA3.1-WNT5A and G418 selection in K562. The number of G418-resistant colonies in the control vector–transfected cell line was set to 100%. Mean values plus or minus SD of 3 separate experiments are shown.

tumor cell lines including NL (4/4), leukemia (4/4), and Burkitt lymphoma (BL; 6/6) but not in normal lymph node (LN), peripheral blood mononuclear cell (PBMC), and lymphoblastoid cell lines (LCLs; Figure 1C). The *WNT5A* promoter is a typical CpG island (Figure 1B), thus subject to methylation silencing.[6,7] We analyzed its methylation by methylation-specific PCR (MSP) and detected methylation in all silenced cell lines (Figure 1C). Detailed methylation analysis with bisulfite genomic sequencing (BGS) confirmed the MSP analysis and revealed a high density of methylated CpG sites in silenced cell lines (Figure 1D). After exposure to Aza, *WNT5A* expression was dramatically increased in silenced cell lines (Figure 1C), further elucidating that methylation directly contributes to *WNT5A* silencing in tumor cell lines. MSP analysis also showed that *WNT5A* was methylated in 50% (5/10) of BL, 73% (22/30) of NL, and 31% (11/36) of other types of non-Hodgkin lymphoma but not in 6 normal lymph nodes, 9 PBMC samples, and normal NK cells. Down-regulation of *WNT5A* was detected in methylated BL samples (Figure 1E). These results demonstrated that *WNT5A* methylation is common and tumor specific in lymphomas.

Further functional studies showed that ectopic expression of *WNT5A* resulted in inhibition of tumor cell growth and clonogenicity (Figure 1F-H), consistent with previous studies that WNT5A inhibited B-cell proliferation and *WNT5A* heterozygous mice developed myeloid leukemias and B-cell lymphomas,[5] suggesting that WNT5A could serve as a tumor suppressor.

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Thus, we showed that *WNT5A* is frequently silenced by methylation in hematologic malignancies in a tumor-specific manner. Mutations of *APC* and epigenetic silencing of other Wnt pathway components including *APC, SFRP*s, *DKK*s, and *WIF1* lead to the activation of canonical Wnt/β-catenin signaling. The noncanonical Wnt proteins (such as *WNT5A*), by activating alternative signaling pathways (such as the orphan tyrosine kinase ROR2), inhibit β-catenin stabilization[8,9] or induce Ca^{2+} flux to block canonical signaling downstream through inhibiting TCF-mediated transcription,[5,10] also preventing inappropriate signaling of the canonical Wnt pathway. WNT5A silencing might act as an additional mechanism to activate the canonical Wnt-signaling pathway. As targeting the Wnt/β-catenin signaling pathway is an attractive therapeutic strategy, our findings also indicate a possibility that *WNT5A* might be used as a therapeutic target.

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Contribution: J.Y., G.S., and Q.T. designed and analyzed data; J.Y. and Q.T. wrote the paper; Z.G. contributed vital samples; and J.Y., H.L., and Y.-W.C. performed research and analyzed data.

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To the editor:

Rituximab as retreatment for rituximab pretreated HIV-associated multicentric Castleman disease

Rituximab is active as initial treatment for HIV-associated multicentric Castleman disease (HMCD). Its efficacy and safety in rituximab pretreated, relapsed patients has not been previously described. We retreated a series of patients with rituximab at histologically confirmed relapse, after they had initially responded with a sustained clinical and radiologic response including normalization of Kaposi sarcoma-associated herpesvirus (KSHV) levels, C-reactive protein (CRP), albumin, hemoglobin, and lactate dehydrogenase (LDH) within 1 month of completing 4 infusions at weekly intervals. At retreatment we observed further clinical, radiologic, biochemical, haematologic, and virologic responses with rituximab. HMCD retains sensitivity to rituximab, suggesting that relapse may not be due to progression of resistant multicentric Castleman disease (MCD) but due to ongoing lytic KSHV infection of plasmablasts. Rituximab is safe and effective for patients with relapsed HMCD.

There are no established treatments for patients with relapsed HIV-associated multicentric Castleman disease. Because data from other cancers such as follicular lymphoma suggest that retreatment with rituximab may be beneficial, we present the findings of rituximab retreatment of HMCD in 3 patients who previously received this monotherapy (4 cycles of once per week rituximab therapy at a standard dose of 375mg/m^2). The patient characteristics at relapse and before and after retreatment with rituxmab presentation are shown in Tables 1 and 2. All 3 patients had decreases in KSHV viral load (Figure 1), consistent with their clinical, radiologic, and biochemical responses.

We describe, for the first time, the efficacy of retreatment with rituximab for patients with HMCD previously treated with rituximab. All 3 individuals remain in remission for a second time, after being rechallenged with this therapy. The results are encouraging; although follow up is relatively short (5-10 months).

Table 1. Patient characteristics at relapse

Characteristics	Patient 1	Patient 2	Patient 3
Age, y	44	48	52
Duration of HIV prior to diagnosis, mo	0	15	17
Prior AIDS defining illness	Yes (TB)	Yes (KS)	Yes (KS)
Duration of first remission, mo	26	19	26
Duration of follow up after second remission, mo	6	5	10
Previous KS	No	Yes	Yes
Splenectomy after initial treatment	No	No	Yes

TB indicates tuberculosis; and KS, Kaposi sarcoma.

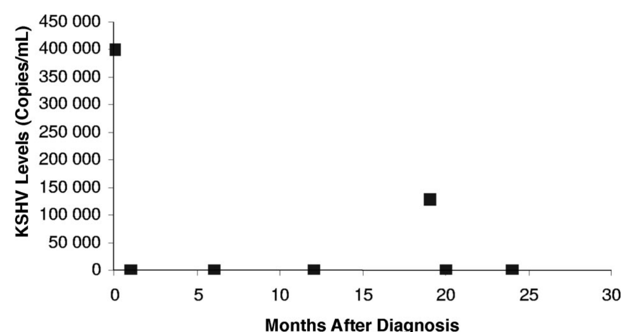

Figure 1. KSHV levels in patient 2. The KSHV levels in this patient demonstrated an elevated viral load at initial presentation (time 0), which declined on 4 cycles of once weekly rituximab, followed by a return of his symptoms and an increase in KSHV viral load (19 months). Once again this elevated viral load declined with rituximab therapy.

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