Oncogene (2004) 23, 6991–6996

www.nature.com/onc

Deregulation of the *carbohydrate (chondroitin 4) sulfotransferase 11* (*CHST11*) gene in a B-cell chronic lymphocytic leukemia with a t(12;14)(q23;q32)

Helmut H Schmidt*[,1], Vadim G Dyomin[1], Nallasivam Palanisamy[1], Takahiro Itoyama[1], Gouri Nanjangud[1], Hendrati Pirc-Danoewinata[2], Oskar A Haas[2] and RSK Chaganti[1]

[1]*Cell Biology Program, and Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY, USA;* [2]*CCRI, Children's Cancer Research Institute, St Anna Kinderspital, Kinderspitalgasse 6, A-1090 Vienna, Austria*

The t(12;14)(q23;q32) breakpoints in a case of B-cell chronic lymphocytic leukemia (B-CLL) were mapped by fluorescence *in situ* hybridization (FISH) and Southern blot analysis and cloned using an *IGH* switch-γ probe. The translocation affected a productively rearranged *IGH* allele and the carbohydrate (chondroitin 4) sulfotransferase 11 (*CHST11*) locus at 12q23, with a reciprocal break in intron 2 of the *CHST11* gene. *CHST11* belongs to the HNK1 family of Golgi-associated sulfotransferases, a group of glycosaminoglycan-modifying enzymes, and is expressed mainly in the hematopoietic lineage. Northern Blot analysis of tumor RNA using *CHST11*-specific probes showed expression of two *CHST11* forms of abnormal size. 5′- and 3′-Rapid Amplification of cDNA Ends (RACE) revealed *IGH/CHST11* as well as *CHST11/IGH* fusion RNAs expressed from the der(14) and der(12) chromosomes. Both fusion species contained open reading frames making possible the translation of two truncated forms of CHST11 protein. The biological consequence of t(12;14)(q23;q32) in this case presumably is a disturbance of the cellular distribution of CHST11 leading to deregulation of a chondroitin-sulfate-dependent pathway specific to the hematopoietic lineage.
Oncogene (2004) **23,** 6991–6996. doi:10.1038/sj.onc.1207934
Published online 26 July 2004

Keywords: B-CLL; translocation; carbohydrate (chondroitin 4) sulfotransferase 11; CHST11; Golgi retention

The molecular analysis of recurring chromosomal translocations has provided valuable insights into the pathobiology of non-Hodgkin's lymphomas (NHL) (Chaganti *et al*., 2000; Willis and Dyer, 2000). However, in B-cell chronic lymphocytic leukemia (B-CLL), specific molecular mechanisms underlying characteristic cytogenetic abnormalities remain for the most part unknown. *IGH* gene-associated translocations that led to the identification of a number of oncogenes involved in NHL are infrequent in B-CLL (Chaganti *et al*., 2000; Willis and Dyer, 2000). Cloning of two recurring t(14;19)(q32;q13) and t(2;14)(p13;q32) translocation breakpoints has led to the identification of the *BCL3* and *BCL11A* oncogenes, respectively, which are deregulated in small subsets of B-CLL (McKeithan *et al*., 1987; Satterwhite *et al*., 2001). The most common abnormalities in B-CLL are del(13q14) and +12. While much work has focused on the identification of a candidate tumor suppressor gene at 13q14, the molecular consequences of trisomy 12 are completely unclear (Chaganti *et al*., 2000). A gene dosage effect has been postulated, but so far, no specific genes have been implicated in this abnormality. Previous studies on partial trisomy of chromosome 12 suggested pathogenetic importance of the segment q13–q22 (Gahrton *et al*., 1982). We report here the molecular cloning of the breakpoints in a t(12;14)(q23;q32) translocation from a case of B-CLL that led to the disruption of the *carbohydrate (chondroitin 4) sulfotransferase 11* (*CHST11*, also known as *chondroitin-4-O-sulfotransferase)* gene (Hiraoka *et al*., 2000; Okuda *et al*., 2000), and formation of reciprocal *IGH-CHST11* and *CHST11-IGH* fusion transcripts. *CHST11*, predominantly expressed in hematopoietic cells, is a member of the HNK1 family of genes that encode Golgi-associated sulfotransferases that are involved in the biosynthesis of glycosaminoglycans (GAG). Both fusion RNAs from the translocation detected in the tumor cells can potentially be translated into truncated proteins and may lead to deregulation of CHST11 protein trafficking across intracellular membranes, possibly resulting in disturbance of the intracellular distribution of CHST11.

Case report

A Caucasian male, born in 1943, was diagnosed with B-CLL in 1984. The tumor cells were positive for CD5, CD19, CD20, CD23, CD38 and IgM. G-banding analysis of the peripheral blood tumor cells revealed the karyotype: 46, XY, t(12;14)(q23;q32). The patient

*Correspondence: HH Schmidt, Department of Internal Medicine, Division of Hematology, University Medical Center, Auenbruggerplatz 38, A-8036 Graz, Austria;
E-mail: helmut.schmidt@klinikum-graz.at
Received 29 February 2004; revised 10 May 2004; accepted 10 May 2004; published online 26 July 2004

had elevated but stable blood counts (WBC $>100\times10^9$/L) and splenomegaly without requirement of chemotherapy. Cytogenetic studies in 1998 following disease progression revealed the karyotype: 46, XY, t(12;14)(q23;q32), del(13)(q14). The patient was resistant to chemotherapy and succumbed to his disease a few months later.

The exact position of the breakpoints was determined by fluorescence *in situ* hybridization (FISH) and Southern blot analysis. Multiple DNA rearrangements observed in the index case suggested that both *IGH* alleles were affected. Since the tumor expressed IgM, one of the *IGH* alleles was assumed to be involved in a productive VDJ rearrangement. A genomic λGem11 library of the tumor DNA was constructed and screened with Jh- and Cμ probes. Sequence analysis of Jh- and Cμ-positive phage clones revealed a nonfunctional DJ rearrangement and a deletion from exon 3 of Cμ to switch-γ*1* of the same allele (data not shown). These rearrangements, presumably due to erroneous DJ- and switch recombination events, characterized the nonfunctional allele. FISH signals with a Jh–Cμ probe were seen on the normal chromosome 14, the der(12) and der(14) chromosomes, whereas the Cγ probe hybridized to the normal chromosome 14 and the der(14) chromosome, yielding only inconsistent signals on the der(12) chromosome (Figure 1). These data indicated that the breakpoint was within the *IGH* gene downstream of *Jh*/*C*μ and upstream of *C*γ*1* or *C*γ*3*.

A 5.5 kb *Hind*III rearrangement detected by the 5′switch γ (Sγ) probe was cloned by screening a genomic λGem11 tumor library. Phage clone 305B hybridized with the 5′Sγ but not the Cγ probe, and, by FISH analysis, was shown to contain sequences specific for chromosomes 12 and 14 (Figure 2). Sequence analysis confirmed the breakpoint on der(12) (accession number AY533201) (Figure 2). To clone the der(14) breakpoint by polymerase chain reaction (PCR), a primer pair was designed such that the forward primer was based on the published chromosome 12 germline sequences (accession number AC079316) telomeric to the breakpoint, and the reverse primer was derived from the Sγ1 sequence (accession number U39737). A 1.6 kb fragment was amplified from the tumor DNA but not from placental DNA. The PCR product revealed sequences colinear with Sγ1 and germ line chromosome 12, confirming the reciprocal der(14) breakpoint (accession number AY533202). A 9-bp deletion of chromosome 12 sequence and a 6-bp deletion of Sγ1 sequence were found in the immediate vicinity of the breakpoint (Figure 2). Southern blot analysis of the index tumor failed to detect germline bands for both switch-γ1 and Cγ1 regions, indicating rearrangements in both alleles

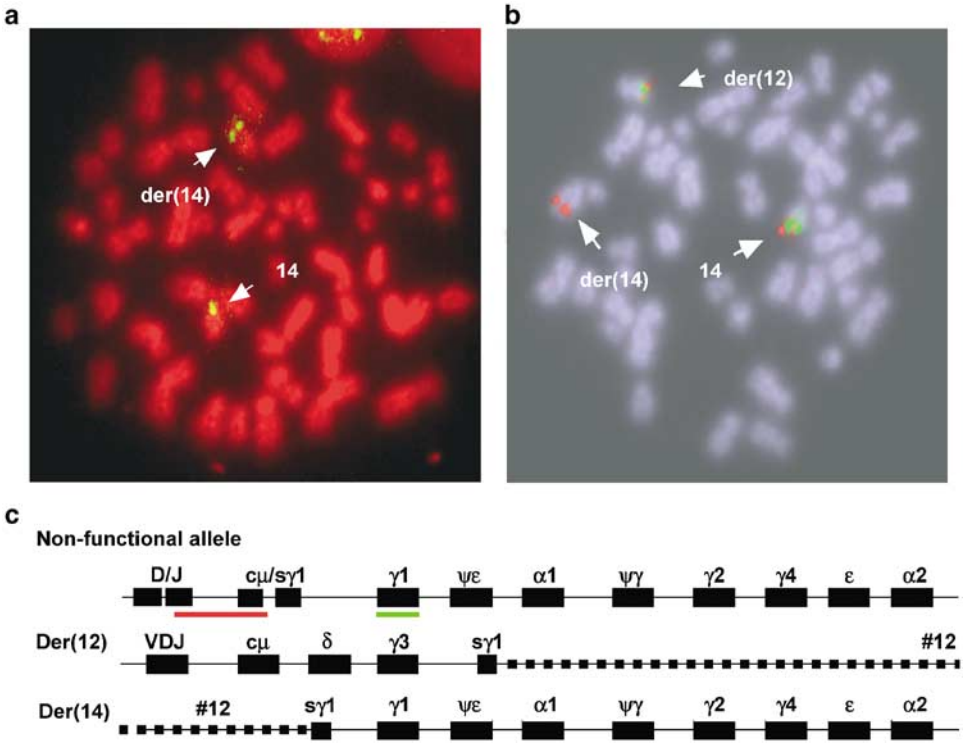

Figure 1 FISH mapping of the t(12;14)(q23;q32) breakpoints. (**a**) Cγ probe hybridizing to the normal chromosome 14 and the der(14) chromosome. (**b**) The Jh–Cμ probe (red) yielded signals on the normal chromosome 14 and the der(12) and der(14) chromosomes, the green probe is a PAC clone comprising 14q telomeric sequences. The signal of the Jh–Cμ probe on the der(14) chromosome presumably was due to crosshybridization of switch-μ sequences contained in the probe with highly homologous switch-α sequences on der(14). (**c**) The genomic organization of both IgH alleles from the index case, the translocation occurred in the functional allele. The colored lines indicate the genomic derivation of the Jh–Cμ and Cγ probes, the Cγ probe recognizes all γ – regions including $\psi\gamma$

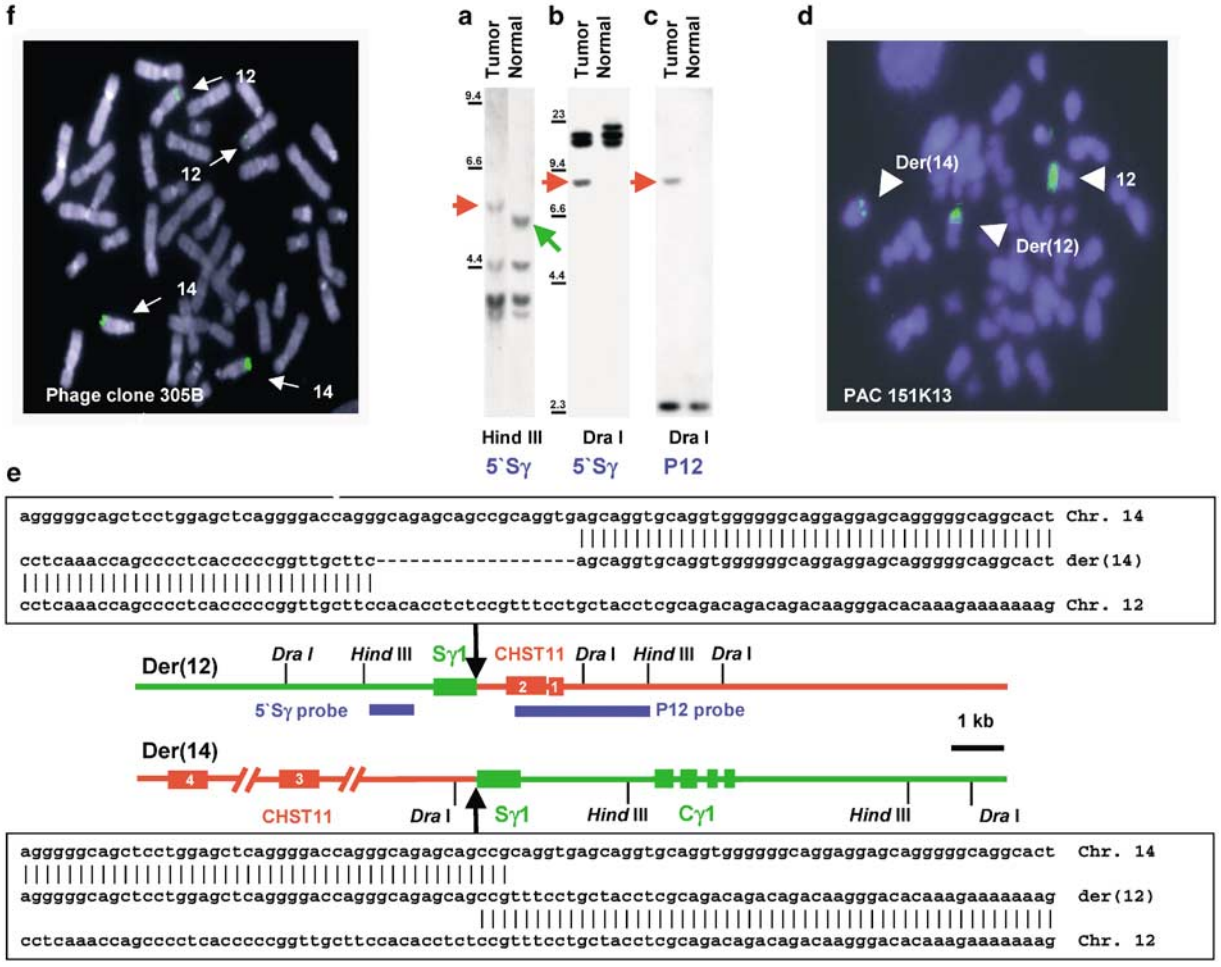

Figure 2 Cloning of the t(12;14)(q23;q32) breakpoints. (**a**) DNA rearrangement (red arrow) detected by hybridization of the 5′switch-γ (5′Sγ) probe to *Hind*III digests of the index tumor DNA, the green arrow indicates the germline band of the switch γ1 region in the normal control DNA. (**b, c**) Co migration of *Dra*I rearranged bands detected by the 5′Sγ probe and p12 probe (chromosome 12-derived DNA fragment of the breakpoint phage clone). (**d**) FISH analysis of the index tumor metaphase spreads. Chromosome 12-derived PAC clone 151K13 shows hybridization signals on chromosomes 12, der(12) and der(14). (**e**) Maps of the breakpoint regions and breakpoint sequences of the derived chromosomes. (**f**) Splitting signal of breakpoint phage clone 305B on normal chromosomes 12 and 14. The *IGH* gene probes (Jh, Cμ, Cγ, Cα, Cε) and hybridization protocols used for FISH, Southern and Northern blotting and genomic library screening have been described elsewhere (Rao *et al*., 1993; Chen *et al*., 1998; Dyomin *et al*., 2000). The 5′Sγ probe was amplified by PCR from genomic DNA as previously described (Bergsagel *et al*., 1996). A genomic library of the tumor DNA was constructed in λGEM-11 phage vector (Promega, Madison, WI, USA) by partial *Sau*3A digestion, and was screened using the probes detailed above. The chromosome 12 PAC clone 151K13 was isolated by PCR-based screening of a PAC library (Genome Systems, St Louis, MO, USA) using the primer pair 5′-TCCAAATTAATACAGCGCACAC-3′ and 5′-TATCCTCCCCTCGATAGACAGA-3′. Primers used for cloning of the der(14) breakpoint comprised 5′-ACCACTCTCCACACCTGCTC-3′ (chromosome 12) and 5′-GCTCAGTCACCACAACCTCA-3′ (chromosome 14)

(Figure 2a and data not shown). Owing to the above-described Cμ-exon 3/switch-γ1 deletion, the 5′-switch γ1 region of the nonfunctional allele was missing, this was confirmed by Southern blot analysis of the corresponding phage clones (data not shown). As the der(12) breakpoint was cloned with a 5′Sγ- probe, the translocation must have occurred in the functional allele of the IgM+ tumor (Figure 1). A chromosome 12 derived PAC, 151K13, hybridized to the normal chromosome 12 as well as to the der(12) and der(14) on tumor metaphases (Figure 2). Genbank data search identified exon 2 of the *CHST11* gene (accession number NM_018413) within 300 bp of the breakpoint. *CHST11* has been mapped to 12q23 (Hiraoka *et al*., 2000; Okuda *et al*., 2000) and consists of four exons spanning a genomic region of 302 kb (Venter *et al*., 2001), the ATG start codon is located in exon 2. CHST11 protein is a Golgi-associated sulfotransferase consisting of a small cytosolic domain and a type II transmembrane region encoded by exon 2 followed by an intraluminal catalytic domain encoded by exons 3 and 4. Northern blot analysis using a full-length *CHST11* cDNA probe (IMAGE clone 2388199) showed, consistent with previous results (Hiraoka *et al*., 2000; Okuda *et al*., 2000), approximately 6.0, 5.0 and 2.0-kb transcripts that were observed in the index case, peripheral blood

mononuclear cells (PBMNC) (Figure 3) and B- and T-cell tumor cell lines (data not shown). An additional 1.7-kb transcript was observed only in the index tumor RNA (Figure 3). Northern blot analysis using the p12 genomic fragment containing exon 1 and the major part of exon 2 revealed an approximately 1 kb band in the index case but not in the PBMNC control (Figure 3). 5′-Rapid Amplification of cDNA Ends (RACE) using gene-specific primers in exon 4 yielded two different species of *IGH-CHST11* fusion cDNA. Notably, in both cases, the transcription started in Cγ1 region in the direction opposite to that of normal *IGH* transcription. *IGH* sequences approximately 130 bp in size – exon 4 of Cγ1 (Fusion RNA I, accession number AY533205) or sequence 0.5 kb 3′ of exon 4 of Cγ1 (Fusion RNA II, accession number AY533204) – were followed in both fusions by exons 3 and 4 of *CHST11* (Figure 3). In the *IGH* part, none of the fusion species contained an open reading frame longer than 36 bp. As exon 3 of *CHST11* starts with an in-frame ATG codon, it is possible that a truncated form of CHST11 protein retaining the catalytic domain, but lacking the N-terminal cytosolic and transmembrane domains, is expressed.

A reciprocal *CHST11–IGH* fusion (Fusion RNA III, accession number AY533203) was detected in a 3′-RACE experiment. The major part of exon 2 of *CHST11* was joined to a 700 bp *IGH*-derived sequence originating from Cγ3–Cγ1 intervening region, the last 15 bp of *CHST11* exon 2 were missing presumably due to alternative splicing, no mutations or deletions were found in exon 2 or the splice donor site by sequence analysis of genomic tumor DNA. The fusion RNA (Figure 3) contained an in-frame stop codon 120-bp 3′ of the RNA junction and an alternative polyadenylation signal (TATAAA) 23-bp upstream of the poly-A tail, suggesting possible translation of a 69 aa fusion protein comprising 34 aa of *CHST11* (the cytosolic domain and the major part of the transmembrane domain) fused to a 35 aa *IGH*-derived part. All RNA fusion species were confirmed in reverse transcriptase (RT)–PCR experiments using primers from different chromosomes. Reaction products of the expected size were recovered only in the index case but not in PBMNC. An internal control reaction with primers specific for exons 2 and 4 of *CHST11* yielded a band of the expected size in both the index case and in PBMNC (data not shown). Interphase FISH analysis of 50 B-CLL cases using the RPCI-11 BAC clones 320O18 (*CHST11* exon 1 and 2) and 381P24 (*CHST11* exon 4), as well as Southern blot analysis of a panel of 20 B-cell NHL cases with 12q22-24 cytogenetic abnormalities using a full-length *CHST11* cDNA probe (IMAGE clone 2388199) and a 4 kb

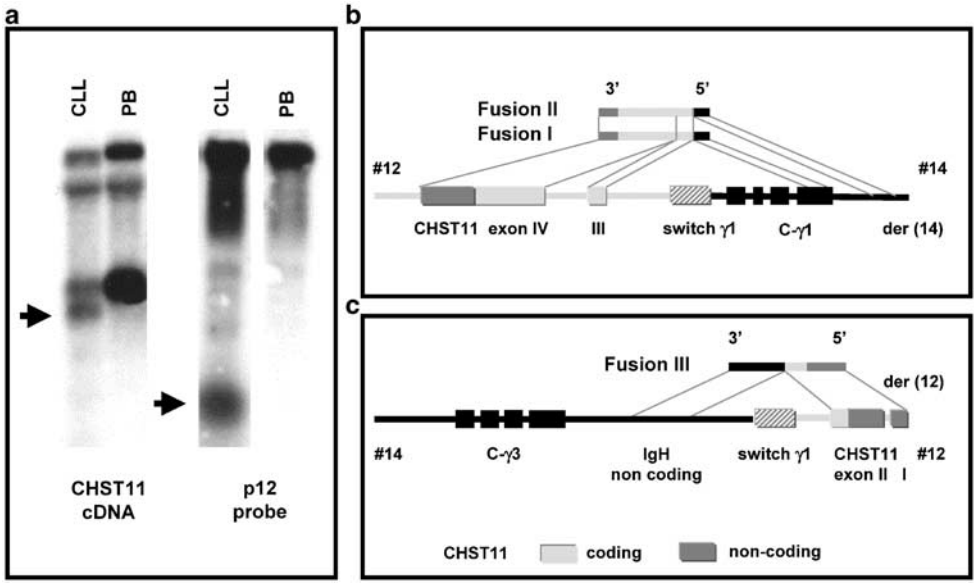

Figure 3 Aberrant expression of *CHST11* gene in the index case. **(a)** Northern blot analysis of the tumor RNA with a full-size *CHST11* cDNA probe and the p12 probe containing exon 1 and the major part of exon 2 showing bands of abnormal size. **(b,c)** Maps of the breakpoint regions on der(12) and der(14) showing derivation of fusion RNA species I–III. 5′- and 3′-RACE experiments were performed according to the manufacturer's instructions (Life Technologies, Rockville, MD, USA). RT–PCR and 5′- and 3′-RACE were performed using 2.5 μg of DNAse-treated tumor RNA purified with RNEasy Kit (Qiagen, Valencia, CA, USA). Primers specific for exon 4 of *CHST11* used for 5′-RACE comprised: 5′-GTTCAGGGTCTTCAGGT-3′ for first strand cDNA synthesis, 5′-ACCTTGGGCACGTAGCAGTAGATG′-3′ and 5′-GTAGCAGTAGATGAGCTCGTGGT-3′ for PCR amplification. First strand synthesis reactions for 3′-RACE were primed with oligo-dT primer (Life Technologies, Rockville, MD, USA). *CHST11* exon 2-specific primers 5′-GCTGCTGGAAGTGATGAGGATGA-3′ and 5′-TTGCTTGGGATCCTTTATCCTGG-3′ were used in subsequent PCR reactions. Primers for detection of the fusion RNA products by RT–PCR comprised: 5′-GGAGGCGTGGTCTTGTAGTT-3′ (*IGH*) and 5′-CTGGATTGGGTTGTAGAGTTCCT-3′ (*CHST11*) for fusion I, 5′-GTGGAGAAGCTGCCGAGA-3′ (*IGH*) and 5′-CTGGATTGGGTTGTAGAGTTCCT-3′ (*CHST11*) for fusion II, 5′-TTGCTTGGGATCCTTTATCCTGG-3′ (*CHST11*) and 5′-GCTTTGTCCTTGCCTGAGTC-3′ (*IGH*) for Fusion III. *CHST11*-specific internal control primers were: 5′-TGCTGGAAGTGAT-GAGGATG-3′ and 5′-GTTCAGGGTCTTCAGGT-3′

genomic *Hind*III fragment (H416) comprising *CHST11* exon I and II did not identify additional rearrangements (data not shown).

The biologic roles of proteoglycans have remained underestimated for a long time (Perrimon and Bernfield, 2000; Selleck, 2000). However, recent genetic studies in Drosophila and mouse provided evidence that glycosaminoglycan-modifying enzymes play crucial roles in developmental processes and growth regulation (Hacker *et al*., 1997; The *et al*., 1999). They have also been implicated in human malignancy: hereditary multiple exostoses (HME), a syndrome characterized by growth abnormalities and tumor susceptibility, is caused by mutations in several *EXT* genes, a family of heparan sulfate copolymerases (McCormick *et al*., 1998; Duncan *et al*., 2001). These data underscore the highly specific cellular functions of proteoglycans and their modifying enzymes. CHST11 is a new member of this functional group of proteins; its predominant expression in the hematopoietic system suggests that it specifically acts on a yet to be identified hematopoietic proteoglycan, also the functional impact of sulfation of this target protein remains to be determined. It is noteworthy that the leukocyte-specific cell adhesion molecule CD44 has been shown to both contain and interact with chondroitin sulfate (Naujokas *et al*., 1993; Greenfield *et al*., 1999), furthermore CD44 activation depends on sulfation (Maiti *et al*., 1998). On the protein level, CHST11 shows 31% homology to the HNK-1-sulfotransferase (HNK1-ST), so far the best-investigated member of this gene family. HNK1-ST adds sulfate to the HNK-1 epitope (CD 56), which was initially described on human natural killer cells but is also found as a carbohydrate decoration of cerebral cell adhesion molecules like neural cell adhesion molecule (NCAM) and others (Ong *et al*., 1998). Functional studies indicated that the HNK-1 glycan is involved in neural cell–cell and cell–substratum interactions and its activation depends on sulfation, which suggests a possible homologous pathway in the hematopoietic system triggered by the CHST11 protein (Ong *et al*., 1998).

GAG-modifying enzymes are considered to reside in the endoplasmic reticulum/Golgi in a spatial configuration, which allows the sequence-specific addition of carbohydrates on proteoglycans that traverse the secretory organelles on their way to the cell surface. Homo- and heterodimerization appear to be important for function and localization, although the mechanisms of Golgi retention of these enzymes are not fully understood (Burke *et al*., 1994; Milland *et al*., 2002). Available experimental evidence suggests that the cytosolic, transmembrane and intraluminal catalytic domains of these enzymes all contribute to their exact localization. Protein leakage to the cell surface has been observed in hybrid molecules where one of these domains had been partially replaced (Burke *et al*., 1994). In addition, constructs expressing only the cytoplasmic tail of some Golgi-associated transferases can prevent the correct localization and abrogate the function of the full-length enzyme (Milland *et al*., 2002). Thus, the predicted protein of the *CHST11–IGH* fusion III may completely abrogate CHST11 function in the index tumor.

The pathogenetic function of the *IgH–CHST11* fusions I and II remains obscure: Devoid of the cytoplasmic and transmembrane domain, the predicted protein should be unable to localize to the Golgi, experimental data suggest cytoplasmic localization of such constructs containing only the catalytic domain (McCormick *et al*., 1998).

These data strongly suggest that the t(12;14)(q23;q32) translocation in this case led to a deregulation of trafficking of the protein across intracellular membranes and thereby disrupted the homeostasis of a yet to be identified chondroitin sulfate-dependent pathway specific to the hematopoietic lineage. FISH and Southern analysis of additional cases indicated that the CHST11 locus is only infrequently rearranged in B-CLL and NHL. However, investigation of the *EXT* genes involved in HME showed that mutations, by yet unknown mechanisms, can deregulate the function of GAG-modifying enzymes (Duncan *et al*., 2001). Furthermore, as heterodimerization with other Golgi-resident proteins may be important for normal function and localization of CHST11, increased levels of expression of *CHST11* by itself could disturb its homeostasis. Thus, a possible role of *CHST11* in the pathogenesis of B-CLL/NHL and trisomy 12 remains to be investigated.

Acknowledgements
This study was supported by various National Institutes of Health grants (RSK Chaganti) and the Austrian Science Fund Grant J1692 and P15121 (HH Schmidt)

References

Bergsagel PL, Chesi M, Nardini E, Brents LA, Kirby SL and Kuehl WM. (1996). *Proc. Natl. Acad. Sci. USA*, **93,** 13931–13936.

Burke J, Pettitt JM, Humphris D and Gleeson PA. (1994). *J. Biol. Chem.*, **269,** 12049–12059.

Chaganti RSK, Nanjangud G, Schmidt H and Teruya-Feldstein J. (2000). *Semin. Hematol.*, **37,** 396–411.

Chen W, Iida S, Louie DC, Dalla-Favera R and Chaganti RSK. (1998). *Blood*, **91,** 603–607.

Duncan G, McCormick C and Tufaro F. (2001). *J. Clin. Invest.*, **108,** 511–516.

Dyomin VG, Palanisamy N, Lloyd KO, Dyomina K, Jhanwar SC, Houldsworth J and Chaganti RSK. (2000). *Blood*, **95,** 2666–2671.

Gahrton G, Robert KH, Friberg K, Juliusson G, Biberfeld P and Zech L. (1982). *Nature*, **297,** 513–514.

Greenfield B, Wang WC, Marquardt H, Piepkorn M, Wolff EA, Aruffo A and Bennett KL. (1999). *J. Biol. Chem.*, **274,** 2511–2517.

Hacker U, Lin X and Perrimon N. (1997). *Development*, **124,** 3565–3573.

Hiraoka N, Nakagawa H, Ong E, Akama TO, Fukuda MN and Fukuda M. (2000). *J. Biol. Chem.*, **275,** 20188–20196.

Maiti A, Maki G and Johnson P. (1998). *Science*, **282,** 941–943.
McCormick C, Leduc Y, Martindale D, Mattison K, Esford LE, Dyer AP and Tufaro F. (1998). *Nat. Genet.*, **19,** 158–161.
McKeithan TW, Rowley JD, Shows TB and Diaz MO. (1987). *Proc. Natl. Acad. Sci. USA*, **84,** 9257–9260.
Milland J, Russell SM, Dodson HC, McKenzie IF and Sandrin MS. (2002). *J. Biol. Chem.*, **277,** 10374–10378.
Naujokas MF, Morin M, Anderson MS, Peterson M and Miller J. (1993). *Cell*, **74,** 257–268.
Okuda T, Mita S, Yamauchi S, Matsubara T, Yagi F, Yamamori D, Fukuta M, Kuroiwa A, Matsuda Y and Habuchi O. (2000). *J. Biochem. (Tokyo)*, **128,** 763–770.
Ong E, Yeh JC, Ding Y, Hindsgaul O and Fukuda M. (1998). *J. Biol. Chem.*, **273,** 5190–5195.
Perrimon N and Bernfield M. (2000). *Nature*, **404,** 725–728.
Rao PH, Murty VV, Gaidano G, Hauptschein R, Dalla-Favera R and Chaganti RSK. (1993). *Genomics*, **16,** 426–430.
Satterwhite E, Sonoki T, Willis TG, Harder L, Nowak R, Arriola EL, Liu H, Price HP, Gesk S, Steinemann D, Schlegelberger B, Oscier DG, Siebert R, Tucker PW and Dyer MJ. (2001). *Blood*, **98,** 3413–3420.
Selleck SB. (2000). *Trends Genet.*, **16,** 206–212.
The I, Bellaiche Y and Perrimon N. (1999). *Mol. Cell*, **4,** 633–639.
Venter JC *et al*. (2001). *Science*, **291,** 1304–1351.
Willis TG and Dyer MJ. (2000). *Blood*, **96,** 808–822.