The role of a newly identified SET domain-containing protein, SETD3, in oncogenesis

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 doi:10.3324/haematol.2012.066977

Online Supplementary Design and Methods

Southern blot and Northern blot

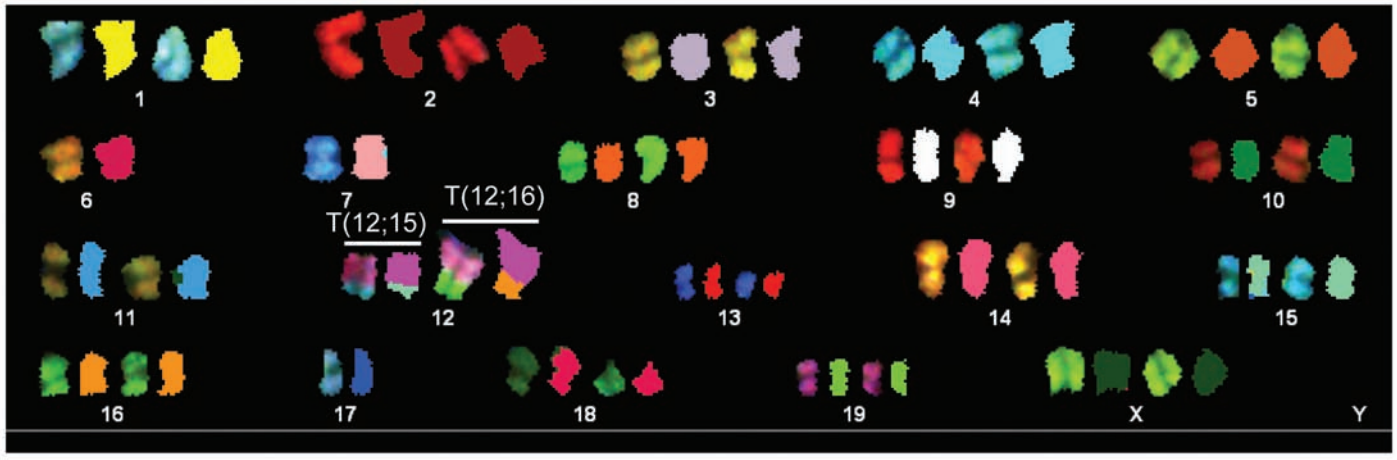
Genomic DNA was isolated from tumor masses or normal tissues from littermate controls, and Southern blotting was performed as previously described.[1] The Cλ1 probe, which has been described previously,[1] hybridizes to Cλ1 and Cλ4 regions due to the sequence homology. RNA samples were extracted from tumor masses or normal tissues from control mice using TriPure Isolation Reagent (Roche). Northern blotting was performed via a standard protocol. Multiple tissue membrane containing total RNA was purchased from Zyagen (San Diego, CA, USA) and membrane containing polyA+ RNA from Sigma (St Louis, MO, USA). The probe for *Setd3* was the cDNA of Setd3-S containing exon 10-13 of the *Setd3-L* gene. A 1.3 kb Pst-I fragment from the GAPDH gene was used as the probe for the GAPDH loading control.

Phage and PCR cloning

Genomic DNA sample from the CXP163 tumor was digested to completion with EcoRI, and fragments were cloned into either lambda ZAP II vector (Stratagene) or lambda DASH II vector (Stratagene) according to the size of the fragments. Libraries were screened according to standard protocols (Stratagene) using the Cλ1 probe for Igλ rearrangements or translocations. Single plaques were purified, subcloned, and sequenced. In the case of lambda ZAPII clones, the inserts were excised according to the manufacturer's protocol (Stratagene). Positive clones were verified by restriction analysis and hybridization. Sequencing of subcloned inserts was performed by a core facility using T7 or T3 primers. To obtain junctional sequences, internal primers were required for subsequent rounds of sequencing.

Expression of SETD3-S and SETD3-L and Western Blot

There are multiple variants of *Setd3* transcripts identified in the database (including NCBI and Ensemble). These include transcripts encoding the full length SETD3-L (594a.a., accession BC019973, *http://www.ncbi.nlm.nih.gov/nuccore/BC019973*), and truncated ones (408a.a., accession BC057968.1, *http://www.ncbi.nlm.nih.gov/UniGene/clust.cgi?ORG=Mm&CID=159185*, and 243a.a., accession AK031017.1, *http://www.ncbi.nlm.nih.gov/nuccore/AK031017*). Full length cDNA of *Setd3* was obtained from ATCC (Genebank: BC010073) and subcloned into the pcDNA.3(+) vector (Invitrogen). A Flag-tag was added to the C-terminal end of cDNAs of *Setd3-S* and *Setd3-L* using PCR. The resulting PCR fragments were subcloned into pcDNA.3(+) (Invitrogen). The



Online Supplemental Figure S1. SKY of CXP163. Karyotype table showing a total of 20 mouse chromosomes. For each individual chromosome, both the spectral image (left) and classified image (right) derived from spectral data are shown. The t(12;16) and t(12;15) translocations are indicated by white lines.

constructs expressing either *Setd3-S* or L or empty vector were transfected into 293T cells using lipofectamine 2000 (Invitrogen). Total cell extracts were prepared from transfected cells and used for Western blot analysis performed with anti-Flag antibody (Sigma).

Cell culture, transfection and infection

293T cells were cultured with RPMI 1640; Phoenix cells, primary MEFs, and NIH3T3 cell were cultured with DMEM. All culture media were supplemented with 10% fetal bovine serum (FBS), antibiotics (Pen/Strep, Invitrogen), 10mM HEPES, and non-essential amino acids (Cellgro). Empty vector or the following vectors (50 μg) were transfected into Phoenix packaging cells via calcium phosphate precipitation. The constructs include pBabe-puro-H-RasVal[12], pBabe-hygro-c-myc, pBabe-puro-*Setd3-S*, and pBabe-puro-*Setd3-L*. A C terminal-HA tag was added to Ras and c-myc cDNA via a PCR approach and the resulting PCR fragments were cloned into pBabe vectors. C terminal-Flag tagged *Setd3-S* and *Setd3-L* were subcloned from pCDNA.3(+) constructs described above. Retroviral supernatants were collected at 48 and 72 h post-transfection and used for infecting NIH3T3 cells. Two days after infection, the infected 3T3 cells were selected under puromycin (4 μg/ml) or hygromycin (150 μg/ml) for 4-5 days and used subsequently for soft agar assay and Western blot analysis.

Soft agar assay

Soft agar assay was performed as described previously.[2] Briefly, the bottom layer of soft agar (Noble Agar, Diffco) was 0.6% in 1×DMEM medium supplemented with 10% FBS. To generate the top layer of soft agar containing cells, logarithmically growing NIH3T3 cells (1×10^4 or 2×10^4) were plated as single cell suspension in 0.4% agar in 1×DMEM supplemented with 10% FBS. Cultures were analyzed and scored after 3-4 weeks of plating. Each cell line was analyzed in quadruplicate. Primary MEFs were freshly explanted (passage zero) as described.[3]

In vitro *HMT assay*

GST, GST-SETD3-S, or GST-SETD3-L were expressed in bacteria and purified via GST sepharose beads for subsequent *in vitro* HMT assay. A HMT assay was performed as previously described.[4] Briefly, for each HMT assay, 2 μg Hela nucleosomes were used. Reactions were carried out at 30°C for 1 h in the presence of [^{3}H]-SAM (S-adenosyl-L-[methyl-^{3}H] methionine). After the *in vitro* HMT reaction, the samples were separated on an SDS-PAGE gel and subjected to autoradiography for three days.

References

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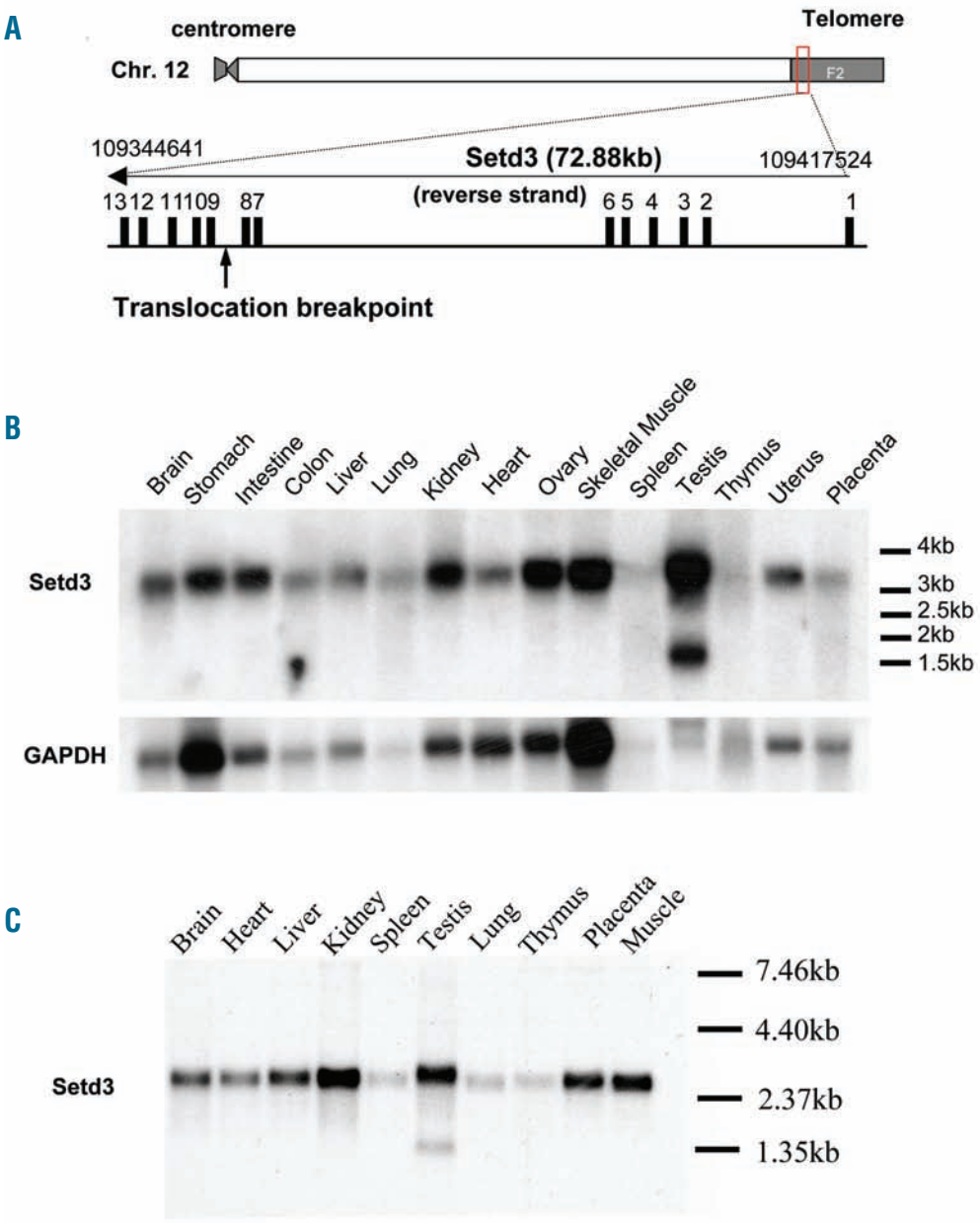
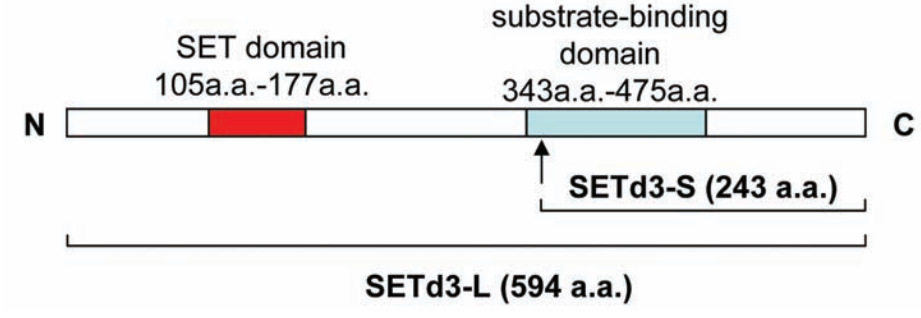


Online Supplemental Figure S2. (A) Detailed locus configuration of *Setd3*. The mouse *Setd3* gene is located on the telomeric end of Chr12 and contains 13 exons (*http://www.ensembl.org/Mus_musculus/Location/View?db=otherfeatures;g=52690;r=12:108106431-108179284;t=NM_028262.3*). The breakpoint of t(16;12;16) is indicated with an arrow. (B) and (C) Characterization of *Setd3* gene expression. Northern blot of multiple tissue membrane from Zyagen (B) or Sigma (C) shows the expression pattern of mouse *Setd3* mRNA in multiple tissues. GAPDH is included as a loading control.



Online Supplemental Figure S3. Schematic map of SETD3 protein. The conserved SET domain (105aa-177aa) is indicated as a red bar. Another conserved domain is the RuBisCo LSMT (lysine methyltransferase) C-terminal, substrate-binding domain (343aa-475aa) (*http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi?seqinput=NP_082538.2*). The start codon of SETD3-S is indicated as a vertical arrow.