Liu et al., http://www.jem.org/cgi/content/full/jem.20072102/DC1

SUPPLEMENTAL RESULTS

Fig. 1 D. A centromeric probe for chromosome 11 revealed three chromosome-11 centromeres in the Granta parent line (A′) and only two in the G4 line (B′). Analysis of the parental and G4 Granta cell lines with a dual-fusion, dual-color *CCND1* (red)/IgH (green) probe revealed three fusion signals (white) in the parental line (C′). Additional green and red signals are from nonrearranged IgH and *CCND1* genes, respectively. Only nonrearranged IgH and *CCND1* genes are detected in the G4 line (D′). In E′ and F′, a centromere probe for chromosome 11 reveals three to four copies in the NCEB parental line (E′), whereas the N1 line (F′) has only two copies of chromosome 11. In G′ and H′, an analysis with the fusion probe from C′ and D′ shows that the NCEB parental cell line has two to four fusions. There is also one normal copy of the *CCND1* region on chromosome 11 (G′). In contrast, the N1 line (H′) has two copies of *CCND1* and three copies of IgH. IgH is rearranged, indicated by the one large green signal (normal IgH) and two smaller green signals (IgH rearrangement), suggesting a loss of the derivative chromosome 11 and rearrangement of IgH on chromosome 14.

SUPPLEMENTAL MATERIALS AND METHODS

Antibodies. The following ChIP antibodies were used: anti-(triacetyl-histone H3) (Millipore), anti-(tetraacetyl-histone H4) (Millipore), CTCF (Millipore), NPM (Santa Cruz Biotechnology, Inc.), Oct-2 (Santa Cruz Biotechnology, Inc.), Bob-1 (Santa Cruz Biotechnology, Inc.), Rel-B (Santa Cruz Biotechnology, Inc.), NF-κB (Santa Cruz Biotechnology, Inc.), MBD1 (Santa Cruz Biotechnology, Inc.), SP1 (Millipore), MeCP2 (Millipore), and MBD2 (Millipore). Cyclin D1 (Santa Cruz Biotechnology, Inc.), cyclin D3 (Sigma-Aldrich), and cyclin D2 antibodies were obtained from M. Welcker (Fred Hutchinson Cancer Research Center, Seattle, WA).

PCR primers. MSP analysis: primers used for the cyclin D1 promoter were 5′-GTAGGCGCGGCGTTTTAG-3′ (−197 to −180 nt relative to the transcription start) and 5′-GCTCGACTCTCGCTTCTAC-3′ (90 to 108 nt); and primers used for the UP3K were 5′-GTCGTTGGCGAAAGGTCG-3′ (−2,827 to −2,810 nt) and 5′-TCATTCAAAAACCGACGCG-3′ (−2,722 to −2,710 nt).
RFLP analysis: primers used were 5′-GTGAAGTTCATTTCCAATCCGC-3′ and 5′-GGGACATCACCCTCACTTAC-3′ for ChIP DNA, or 5′-GAACAAACAGATCATCCGCAA-3′ and 5′-TGCTCCTGGCAGGCACGGA-3′ for cDNA.

Table S1. Summary of FISH results for the presence of t(11;14) in Granta, NCEB, U266, and derivative mutant cyclin D1$^-$ cell lines

	no. of copies of chromosome 11[a]			t(11;14)[b]
Cell line	2	3	4	Positive/negative
LT-neg control	100%			Neg
Granta	11.5%	83.5%		Pos
G4 (D1$^-$)	93.5%	2.5%		Neg
LG1 (D1$^-$)	72.5%	12.5%	10.5%	Neg
LG2 (D1$^-$)	83.5%	8.5%	5.9%	Neg
NCEB	4.5%	39.5%	51.5%	Pos
N1 (D1$^-$)	96%	3%		Neg
N2 (D1$^-$)	99%			Neg
N3 (D1$^-$)	99%			Neg
U266	99.5%	0.5%		Pos
LU1 (D1$^-$)	97.5%	0%	2.5%	Neg
LU2 (D1$^-$)	96%	3.5%	0.5%	Neg

Two probes were used: a centromeric chromosome 11 probe (Oncor) to determine the number of copies of chromosome 11 present, and an 11;14 fusion probe (IgH/*CCND1*; Vysis, Inc.). The diploid fibroblast line LT was used as a negative control. The data showed the loss of the 11;14 chromosome in the mutant cyclin D1$^-$ cell lines. Some NCEB cells had more than one t(11;14), with 23% 2 fusion, 57.8% 3 fusion, and 17.6% 4 fusion. 200 cells were scored per cell line (see Materials and methods).

[a]Chromosome 11 probe to determine the number of copies of chromosome 11.

[b]11;14 fusion probe (IgH/*CCND1*).