

Supporting Information

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SI Materials and Methods

Assays for Growth Inhibition, Cell Death, and DNA Content. Cell proliferation was determined with a modified MTT assay by using a Cell Counting Kit-8 (Dohjindo). Cell death was defined in terms of the intracellular incorporation of propidium iodide as determined by flow cytometry. For DNA content analysis, cells were fixed with ice-cold 70% ethanol, stained with propidium iodide, and then analyzed with flow cytometry. The percentage of the sub-G1 fraction, which was assumed to be undergoing apoptosis, was determined by means of CellQuest software (BD).

Chemotactic Cell Migration Assay. The cell migration assay was performed in 12-well chemotaxis chamber plates (Corning). Cells (5.0×10^5) in serum-free medium were seeded into the upper chambers, and serum-free medium or CM/HS-5 was added to the lower chambers. The number of cells that had transmigrated into the lower chamber was counted with a FACSCalibur flow cytometer with appropriate gating for 5 min at a high flow rate after 3, 6, and 24 h incubation. The experiments were performed in

triplicate. The number of cells that had transmigrated to the serum-free medium after 3 h incubation was assigned a value of 1.0.

Measurement of Gal-3 Concentration in Media. Gal-3 concentrations of CM/MYL, CM/MYL/G3, CM/K562, and CM/K562/G3 were measured by using Human Galectin-3 Assay Kit (Immuno-Biological Laboratories) according to manufacturer instructions.

Western Blot Analysis. Primary Abs used were those against Actin (Sigma-Aldrich), Gal-3 (clone A3A12), Mcl-1 (Santa Cruz Biotechnology), Bcl-2 (clone 100; Upstate Biotechnology), Bad, Bcl-X_L (Stressgen), Bim (3C5; gift from Anderas Strasser, Walter and Eliza Hall Institute, Victoria, Australia), Akt, Erk, phospho (p)-Akt, p-Bad^{Ser112}, and p-Erk (Cell Signaling Technology).

Immunohistochemical Staining. Approval was obtained from the institutional review board at Kyoto Prefectural University of Medicine for a study using patient-derived samples. Formalin-fixed and paraffin-embedded tissues were examined by staining with anti-Gal-3 monoclonal Ab.

Table S1. Genes modified by adhesion to FN and coculturing with HS-5 in MYL cells

Type	Genes up-regulated by >2 folds	Genes down-regulated by <2 folds
Accumulation of cells	BCL3, CFLAR, CR1, CXCL2, EGR1, ICAM1, IER3, IL8, IL1B, OSM, PLAU, TNFSF10, VEGFA	—
Cell adhesion	AXL, CD48, CD55, CD97, CR1, CXCL2, EGR1, ICAM1, IL8, IL1B, GAL-3, LOXL2, MMP15, NEDD9, NTRK1, OSM, PIK3CA, PLAU, PPAR, S1PR1, SLC3A2, TFPI, TGFB11, VEGFA	AKT2, ITGB1
Apoptosis	ADM, AKAP12, ASNS, ATF3, AXL, BCL3, BEX2, BHLHB2, BTG1, CAV1, CD48, CD55, CD69, CEBPB, CFLAR, CR1, CSTA, CTH, CXCL2, DDIT4, DNASE2, DUSP6, EGR1, FOXO1, GGT1, HERPUD1, ICAM1, IER3, IL8, IL15RA, IL1B, IL2RA, LAIR1, GAL-3, LOXL2, MYCT1, NDRG1, NEDD9, NFKBIZ, NTRK1, OSM, PAX6, PCGF2, PIK3CA, PIM2 (includes EG:11040), PLAU, PLK2, PPAR, PRF1, PTAFR, PTPRE, RHEB, S1PR1, SAT1, SLC2A3, SOCS3, TBXA2R, TGFB11, TNFRSF10B, TNFRSF10D, TNFSF10, TRA α , TRIB3, UACA, VEGFA, WNT11, XBP1	AKT2, ARHGDI, ATF5, BRF1, CALR, CDC25A, CDK6, CEBPA, COL2A1, CTBP1, GALNT10, HMGA1, ID3, IL9R, ING5, IRAK1, ITGB1, MPRIP, NF2, PA2G4, PAK2, PDCD6, POLR2A, PRDM2, PRKACA, SPN, SRF, TCF3, TXN, YY1
Cell cycle progression	ASNS, ATF3, CAV1, CCNG2, EGR1, FOXO1, HBP1, HPGD, IER3, IL8, IL1B, IRF9, MX11, OSM, PAX6, PCGF2, PLK2, S1PR1, TNFSF10, VEGFA, XBP1	ARID1A, ATF5, CALR, CDC25A, CDK6, CEBPA, ID3, ITGB1, NUCKS1, PA2G4, SMARCA4, TCF3, TRRAP, YY1
Cell cycle arrest	—	ARID1A, CALR, CDC25A, CDK6, CEBPA, ITGB1, PA2G4, SMARCA4, TCF3, YY1
Cell division arrest	—	ARID1A, ATF5, CALR, CDC25A, CDK6, CEBPA, ID3, ITGB1, PA2G4, SMARCA4, TCF3, TRRAP, YY1, HMGA1
Cell death	ADM, AKAP12, APOL1, ARAP3, ASNS, ATF3, AXL, BCL3, BEX2, BHLHB2, BTG1, CAV1, CD48, CD55, CD69, CEBPB, CFLAR, CR1, CSTA, CTH, CXCL2, DDIT4, DNASE2, DUSP6, EGR1, FOXO1, GGT1, HERPUD1, ICAM1, IER3, IL8, IL15RA, IL1B, IL2RA, LAIR1, GAL-3, LOXL2, MYCT1, NDRG1, NEDD9, NFKBIZ, NTRK1, OSM, PAX6, PCGF2, PIK3CA, PIM2 (includes EG:11040), PLAU, PLK2, PPAR, PRF1, PTAFR, PTPRE, RHEB, S100P, S1PR1, SAT1, SLC2A3, SOCS3, TBXA2R, TGFB11, TNFRSF10B, TNFRSF10D, TNFSF10, TRA α , TRIB3, UACA, VEGFA, WNT11, XBP1	AKT2, ARHGDI, ATF5, BRF1, CALR, CDC25A, CDK6, CEBPA, COL2A1, CTBP1, GALNT10, HMGA1, ID3, IL9R, ING5, IRAK1, ITGB1, MPRIP, NF2, PA2G4, PAK2, PDCD6, POLR2A, PRDM2, PRKACA, RAD51L3, SPN, SRF, TCF3, TIMM50, TXN, UBTF, YY1
Cell movement	ADM, AXL, CAV1, CD48, CD55, CD69, CD97, CXCL2, ICAM1, IL8, IL1B, IL2RA, GAL-3, MYLIP, NEDD9, NFKBIZ, OSM, PLAU, PRF1, PRKG1, PTAFR, S1PR1, SOCS3, VEGFA	ITGB1
Cell growth	ADM, AKAP12, ATF3, BTG1, CAV1, CD55, CEBPB, CTH, CTSF, CXCL2, DUSP6, EGR1, EIF1, FOXO1, GGT1, HBP1, HNRNP, HPGD, IER3, IFI30, IL8, IL15RA, IL1B, IL2RA, GAL-3, LITAF, MAFF, MMP15, MYCT1, NTRK1, OSM, PCGF2, PIK3CA, PIM2 (includes EG:11040), PLAU, PLK2, S1PR1, SAT1, SLC3A2, SOCS3, TGFB11, TNFSF10, VEGFA, WWTR1, XBP1	AKT2, ATF5, BAT2D1, BRF1, CALR, CBX2, CDC42BPB, CDK6, CEBPA, COL2A1, CTBP1, CYP1A1, EIF4G1, GTPBP1, HMGA1, ID3, IL9R, ITGB1, MAZ, MYBBP1A, NF2, PA2G4, PIP4K2B, PRKACA, SLC19A1, SMARCA4, SOS1, SRF, TCF3, TRRAP, TXN, UBTF
Cell invasion	ADM, ATF3, AXL, CAV1, CD97, ETV1, HBP1, IL8, IL1B, MMP15, NTRK1, OSM, PLAU, S100P, TBXA2R, TNFSF10, VEGFA	AKT2, ITGB1, YY1
Cell migration	ADM, ARAP3, AXL, CAV1, CD69, CD97, CXCL2, EGFL7, EGR1, FOXO1, GNG12, ICAM1, IL8, IL15RA, IL1B, GAL-3, MARCKS (includes EG:4082), NEDD9, OSM, PAX6, PIK3C2B, PLAU, PLXNA1, PPAR, PRKG1, S100P, S1PR1, SEMA4C, SLC3A2, SOCS3, TFPI, TNFSF10, VEGFA, WARS, WNT11, WWTR1	—
Cell morphology	ADM, ATF3, CAV1, CEBPB, EGR1, FOXO1, HBP1, IL1B, GAL-3, MARCKS (includes EG:4082), MYCT1, OSM, PAX6, PRF1, PRKG1, PTPRE, RARRES3, TBXA2R, TGFB11, VEGFA, WWTR1	CDC42BPB, CDC42SE1, GRLF1, IRAK1, ITGB1, MPRIP, NF2, SMARCA4, SOS1, SPN, SRF, TLN1, WASF2
Cell proliferation	ADM, ATF3, AXL, BCL3, BTG1, CAV1, CCNG2, CD48, CEBPB, CFLAR, CR1, CTH, CXCL2, EGR1, ENPEP, FOXO1, GGT1, H19, HLA-E, HPGD, ICAM1, IFI30, IFITM1, IL8, IL15RA, IL1B, IL2RA, KLF9, LAIR1, GAL-3, MX11, NTRK1, OSM, PAX6, PCGF2, PIK3CA, PIM2 (includes EG:11040), PLAU, PPAR, PRKG1, PTAFR, PTPRE, PTPRR, RARRES3, S100P, S1PR1, SAT1, SEMA4C, SLC7A11, SOCS3, TBXA2R, TFPI, TGFB11, TNFRSF10B, TNFSF10, TOM1L1, TRA α , VEGFA, WARS, WNT11	AKT2, ATF5, CALR, CDC25A, CDK6, CEBPA, COL2A1, CTBP1, DDX11, FBRS, HMGA1, HS6ST1, ID3, IL9R, ING5, IRAK1, ITGB1, MAPKAPK2, MYBBP1A, NF2, PA2G4, PATZ1, PIP4K2B, PRKACA, SLC19A1, SMARCA4, SOS1, SPN, SRF, TCF3, TRRAP, TXN, WASF2, YY1

Table S1. Cont.

Type	Genes up-regulated by >2 folds	Genes down-regulated by <2 folds
Transcription	ATF3, BCL3, BHLHB2, BTG1, CAV1, CBY1, CEBPB, CFLAR, CREB3L4, EGR1, ETV1, FOXO1, IL10RB, IL1B, IRF9, KLF9, LITAF, MAFF, MXI1, OSM, PAX6, PCGF2, PLK2, PPAR, PRKG1, PTPRR, RARRES3, S1PR1, SOCS3, TGFB11, TNFRSF10B, TNFSF10, TOM1L1, TRIB3, VEGFA, WWTR1, XBP1	ARID1A, ATF5, BRF1, CALR, CBX2, CCNK, CEBPA, CHD4, CTBP1, EHMT1, EHMT2, FOXK2, GRLF1, HBZ, HMGA1, ID3, IRAK1, ITGB1, LOC339344, MAPKAPK2, MAZ, MLXIP, MYBBP1A, NR2F2, NR2F6, PA2G4, PAK2, PATZ1, PBX2, PHF12, PITX1, POLR2A, PRDM2, PRKACA, RBM14, SLC2A4RG, SMARCA4, SORBS3, SOS1, SPEN, SRCAP, SRF, TCF3, TRRAP, UBTf, YY1, ZNF326

Table S2. Gal-3 expression in patient-derived untreated leukemic cells

Pt. no.	Disease status	ANC ($\times 10^9/L$)	Blast, %	Ph ⁺ cells, %	Gal3 ⁺ cells	Treatment outcome
1	CML-CP	583.0	0.0	99.0	+++	CCyR with IM
2	CML-CP	888.0	0.0	97.0	+++	CCyR with IM
3	CML-CP	916.0	0.8	100.0	+++	CCyR with IM
4	CML-CP	200.0	0.8	95.0	+++	IM intolerance, MMR with Das
5	CML-CP	1375.0	0.4	95.0	+++	CCyR with IM
6	CML-CP	996.0	2.4	99.0	+++	MMR with IM
7	CML-CP	NA	0.8	NA	+++	Failure with IM, Nilo and Das
8	CML-CP	568.0	0.8	100.0	+++	CCyR with IM
9	CML-CP	1968.0	0.4	96.0	+++	IM intolerance, CCyR with Bos
10	CML-CP	NA	2.6	96.0	+++	IM intolerance; MMR with Nilo
11	CML-AP	62.0	18.7	87.0	+++	CCyR with IM
12	CML-LBC	407.0	95.4	98.0	—	Failure with IM+CTx
13	CML-LBC	892.0	85.6	96.0	+	Failure with IM+CTx
14	CML-MBC	780.0	77.5	87.4	+	MMR with IM+CTx
15	CML-MBC	329.0	81.3	98.0	+	HCR with IM+CTx
16	CML-MBC	182.0	29.2	81.0	++	Failure with CTx
17	CML-MBC	458.0	36.1	97.0	++	HCR with IM+CTx
18	CML-MBC	424.0	56.0	95.0	++	HCR with IM+CTx
19	CML-MBC	957.0	51.4	100.0	++	MMR with IM+CTx
20	CML-MBC	907.0	46.8	100.0	+++	PCyR with IM+CTx, relapse
21	Ph ⁺ AML	512.0	28.8	92.0	+++	MMR with IM+CTx
22	Ph ⁺ ALL	706.0	97.8	100.0	—	CMR with IM+CTx
23	Ph ⁺ ALL	NA	86.8	NA	—	CCyR with IM+CTx
24	Ph ⁺ ALL	1581.0	94.2	35.0	+	MMR with IM+CTx
25	Ph ⁺ ALL	704.0	84.6	25.0	—	MMR with IM+CTx
26	AML (M0)	61.0	80.4	NA	+	CR with CTx
27	AML (M3)	359.0	84.4	NA	—	CR with ATRA+CTx
28	AML (M3)	105.0	79.2	NA	+	CR with ATRA+CTx
29	AML (M4)	62.0	25.8	NA	+	CR with CTx
30	AML (M5a)	594.0	84.0	NA	+	Primary refractory to CTx
31	Common ALL (L2)	460.0	87.6	NA	+	CR with CTx
32	Healthy volunteer	123.0	0.0	NA	+	NA
33	Healthy volunteer	50.0	1.2	NA	+	NA
34	Healthy volunteer	144.0	0.8	NA	+	NA

Frequency of Gal-3-positive cells in BM clot sample was scored and represented as follows: —, <5% cells positive for cytoplasmic Gal-3 expression; +, 5–20% cells are positive for Gal-3; ++, approximately 21–50% cells are positive for Gal-3; +++, >50% cells are positive for Gal-3. ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; ANC, all nucleated BM cells; ATRA, all-*trans* retinoic acid; Bos, bosutinib; CCyR, complete cytogenetic response; CR, complete remission; CTx, conventional chemotherapy; MMR, major molecular response; NA, not applicable; Nilo, nilotinib.

Table S3. Gal-3 expression in patient-derived leukemic cells

Pt. no.	Diagnosis	Disease status	ANC ($\times 10^9/L$)	Blast, %	Ph ⁺ cells, %	Gal3 ⁺ cells
19	CML-BC	Onset	957.0	51.4	100.0	++
		First relapse	44.0	2.8	26.0	++
		Second relapse	66.0	72.0	95.0	+
22	Ph ⁺ ALL	Onset	706.0	97.8	100.0	—
		Relapse	71.0	77.8	70.0	+
23	Ph ⁺ ALL	Onset	NA	86.8	NA	—
		Relapse	122.0	52.2	80.0	++
24	Ph ⁺ ALL	Onset	1581.0	94.2	35.0	+
		Relapse	150.0	80.0	87.0	+

Gal-3 expression in leukemic cells at different disease stages was immunohistochemically examined in patients 19, 22, 23, and 24 (Table 1). Frequency of Gal-3-positive cells in BM clot sample was scored and represented as follows: —, <5% cells positive for cytoplasmic Gal-3 expression; +, 5–20% cells are positive for Gal-3; ++, approximately 21–50% cells are positive for Gal-3; +++, >50% cells are positive for Gal-3. ALL, acute lymphoblastic leukemia; ANC, all nucleated BM cells; NA, not applicable.