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. Formalinfixed and paraffin-embedded tissues were examined by staining with anti–Gal-3 monoclonal Ab.

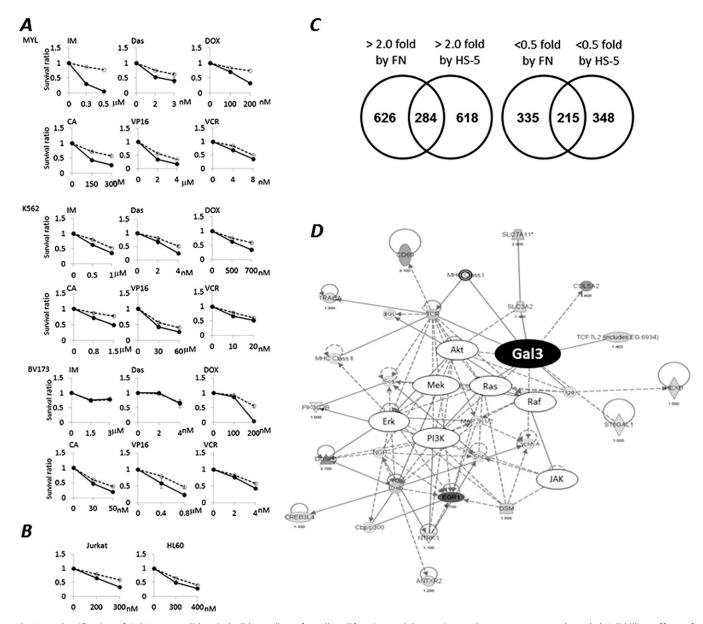


Fig. S1. Identification of Gal-3 as a candidate inducible mediator for cell proliferation and drug resistance by BM components. (A and B) Cell-killing effects of TKIs and genotoxic agents on leukemic cell lines with or without the coculture of HS-5. The x axis indicates the drug concentration and the y axis indicates the cell survival ratio relative to untreated cells after 48 h treatment. Solid lines represent cells without HS-5 and dotted lines represent cells with HS-5. CA, cytosine arabinoside. (A) Ph⁺ cell lines were treated with TKIs or genotoxic agents for 48 h in the presence (dotted lines) or absence (solid lines) of HS-5, and (B) Ph⁻ Jurkat T and HL60 cells were treated with DOX for 48 h in the presence (dotted lines) or absence (solid lines) of HS-5. (C) Numbers of genes significantly modified by two BM components: adhesion to FN and/or coculture with HS-5 in MYL cells. (D) Signal pathway analysis based on gene expression profiles modified by BM components.

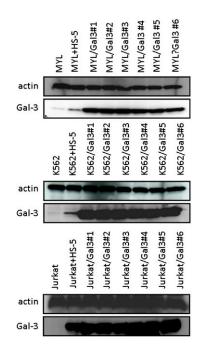


Fig. S2. Generation of Gal-3-overexpressing MYL, K562, and Jurkat T cells. Several clones were developed for each cell line.

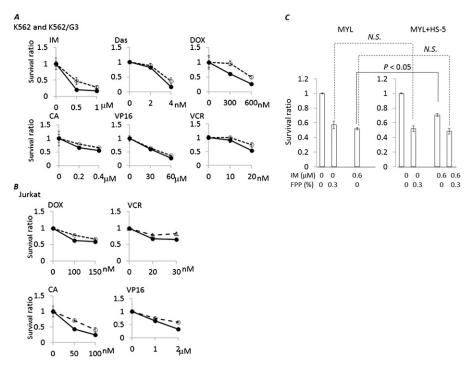


Fig. S3. Cell-killing effects of genotoxic agents. The x axis indicates the drug concentration and the y axis indicates the survival cell ratio after 48 h treatment. (A) Solid lines indicate parental K562 cells and dotted lines indicate K562/G3 cells. (B) Solid lines indicate parental Jurkat T cells and dotted lines indicate Jurkat/G3 cells. (C) MYL cells acquired resistance to IM-induced cell killing by coculture with HS-5, whereas the Gal-3 inhibitor FPP counteracted this scenario.

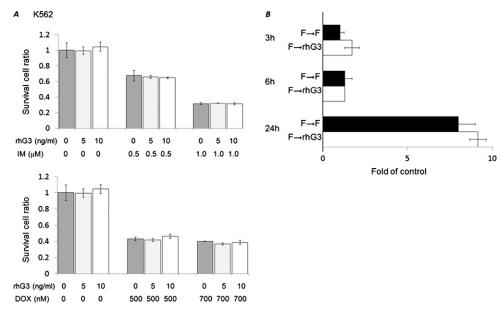


Fig. S4. (A) K562 cells were treated with IM or DOX for 48 h with or without rhGal-3, and were subjected to MTT assays. The addition of recombinant rhGal-3 (5 or 10 ng/mL) did not confer leukemic cells more resistant to cell death by IM or DOX. (B) The addition of rhGal-3 did not promote cell migration of leukemic cells. The number of migrated cells in MYL after 3 h incubation was assumed to be 1.0. F, serum free medium; rhG3, rhGal-3 10 ng/mL; F→rhG3, upper chamber supplemented with serum free medium and lower chamber filled with rhG3.

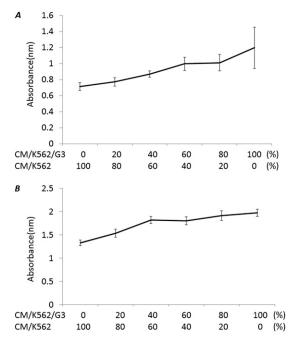


Fig. S5. CM of Gal-3–overexpressing cells contains more growth promoting soluble factors. K562 cells (A) and HS-5 cells (B) were grown together with the mixtures of various concentrations of CM/K562 and CM/K562/G3. Cell proliferation was determined by means of MTT assay. An increase in concentration of CM/K562/G3 promotes cell proliferation of both K562 and HS-5 cells.

Fig. 56. Microscopic view of leukemia involvement (BM plus extramedullary tumor invaded from BM) in mice xenografted with MYL/G3 cells.

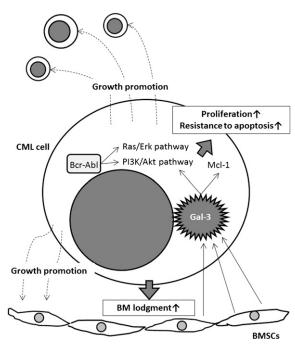


Fig. S7. Proposed model of mechanisms of Gal-3 action in BMME-mediated cell proliferation and drug resistance in BM.

| Туре | 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, PPARD, S1PR1, SLC3A2, TFPI, TGFB1I1, 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, IL18, IL2RA, LAIR1, GAL-3, LOXL2, MYCT1, NDRG1, NEDD9, NFKBIZ, NTRK1, OSM, PAX6, PCGF2, PIK3CA, PIM2 (includes EG:11040), PLAU, PLK2, PPARD, PRF1, PTAFR, PTPRE, RHEB, S1PR1, SAT1, SLC2A3, SOCS3, TBXA2R, TGFB111, TNFRSF10B, TNFRSF10D, TNFSF10, TRAα, TRIB3, UACA, VEGFA, WNT11, XBP1 | AKT2, ARHGDIA, 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, MXI1, 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 | , , , , <u>, </u> | ARID1A, CALR, CDC25A, CDK6, CEBPA, ITGB1, PA2G4, SMARCA4, TCF3, YY1 |
| Cell division arrest Cell death | — ADM, AKAP12, APOL1, ARAP3, ASNS, ATF3, AXL, BCL3, | ARID1A, ATF5, CALR, CDC25A, CDK6, CEBPA, ID3, ITGB1, PA2G4, SMARCA4, TCF3, TRRAP, YY1, HMGA1 AKT2, ARHGDIA, ATF5, BRF1, CALR, CDC25A, CDK6, CEBPA, |
| | 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, PPARD, PRF1, PTAFR, PTPRE, RHEB, S100P, S1PR1, SAT1, SLC2A3, SOCS3, TBXA2R, TGFB1I1, TNFRSF10B, TNFRSF10D, TNFSF10, TRAα, TRIB3, UACA, VEGFA, WNT11, XBP1 | 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, HNRNPD, HPGD, IER3, IF130, IL8, IL15RA, IL1B, IL2RA, GAL-3, LITAF, MAFF, MMP15, MYCT1, NTRK1, OSM, PCGF2, PIK3CA, PIM2 (includes EG:11040), PLAU, PLK2, S1PR1, SAT1, SLC3A2, SOCS3, TGFB1I1, 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, PPARD, 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, TGFB1I1, 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, MXI1, NTRK1, OSM, PAX6, PCGF2, PIK3CA, PIM2 (includes EG:11040), PLAU, PPARD, PRKG1, PTAFR, PTPRE, PTPRR, RARRES3, S100P, S1PR1, SAT1, SEMA4C, SLC7A11, SOCS3, TBXA2R, TFPI, TGFB1I1, TNFRSF10B, TNFSF10, TOM1L1, TRAα, 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 |

Transcription

ATF3, BCL3, BHLHB2, BTG1, CAV1, CBY1, CEBPB, CFLAR, CREB3L4, EGR1, ETV1, FOXO1, IL10RB, IL1B, IRF9, KLF9, LITAF, MAFF, MXI1, OSM, PAX6, PCGF2, PLK2, PPARD, PRKG1, PTPRR, RARRES3, S1PR1, SOCS3, TGFB1I1, 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 (×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 (×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.