ROBO1 Promotes Homing, Dissemination, and Survival of Multiple Myeloma within the Bone Marrow Microenvironment

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ABSTRACT

The bone marrow (BM) microenvironment actively promotes multiple myeloma pathogenesis, and therapies targeting both cancer cells and the niche are highly effective. We were interested in identifying novel signaling pathways supporting multiple myeloma–BM cross-talk. Mutations in the transmembrane receptor Roundabout 1 (ROBO1) were recently identified in patients with multiple myeloma; however, their functional consequences are uncertain. Through protein structure-function studies, we discovered that ROBO1 is necessary for multiple myeloma adhesion to BM stromal and endothelial cells and that ROBO1 knockout (KO) compromises BM homing and engraftment in a disseminated mouse model. ROBO1 KO significantly decreases multiple myeloma proliferation in vitro and intra- and extramedullary tumor growth in vivo. Mechanistically, the ROBO1 C-terminus is cleaved in a ligand-independent fashion and is sufficient to promote multiple myeloma proliferation. Vice versa, mutants lacking the cytoplasmic domain, including the human-derived G674* truncation, act dominantly negative. Interactomic and RNA-sequencing studies suggest that ROBO1 may be involved in RNA processing, supporting further studies.

SIGNIFICANCE: ROBO1 is highly expressed in t(4;14) multiple myeloma and supports homing and dissemination to the BM niche. ROBO1 knockout causes reduced tumor growth in intramedullary and extramedullary myeloma animal models, while the ROBO1 C-terminus is cleaved in multiple fragments and it is necessary and sufficient to sustain myeloma proliferation.

INTRODUCTION

Multiple myeloma is the second most common hematologic malignancy in the Western world (1). Our understanding of molecular mechanisms promoting multiple myeloma pathogenesis and therapy resistance is incomplete, and multiple myeloma remains incurable. Therefore, identification of signaling pathways supporting multiple myeloma proliferation, survival, and resistance to therapy is a priority to improve patient outcomes (2). Progressive dysregulation of the bone marrow (BM) niche with increasing immunotolerant and cancer proproliferative features, is a hallmark of multiple myeloma progression (3, 4). Novel multiple myeloma therapies targeting not only cancerous cells but also the BM niche have proven remarkably effective (5). The overall goal of this study was to identify novel signaling pathways that promote multiple myeloma in the context of its interaction with the BM niche.

ROBO1 is an evolutionarily conserved, transmembrane receptor of the Ig superfamily (6). The extracellular matrix proteins of the SLIT family serve as ROBO1 ligands (7). Initially described as a necessary chemorepellent cue during neuronal development, the SLIT/ROBO axis is genetically and epigenetically altered in cancer, promoting proliferation, metastasis, and angiogenesis (8). ROBO1 has no intrinsic enzymatic activity, and downstream signaling is carried out by recruitment and activation of cadherins, ABL, and β-catenin via four C-terminus, proline-rich domains (9–12). High ROBO1 expression confers poor prognosis in newly diagnosed multiple myeloma, and somatic mutations in ROBO1 have just been described in patients with multiple myeloma at the time of these studies (13–15). Three of these mutations are predicted to result in a truncated protein, devoid of a transmembrane and intracytoplasmic domain. To date, the function of wild-type (WT) ROBO1 activation in multiple myeloma and the functional consequences of the expression of human-derived ROBO1 truncating mutations in multiple myeloma are undefined. In this study, we show that ROBO1 is highly expressed in human multiple myeloma cell lines and primary multiple myeloma cells from patients, especially enriched in t(4;14) multiple myeloma. We discovered that ROBO1 knockdown (KD) is cytotoxic for multiple myeloma, but not other hematologic malignancies. ROBO1 knockout (KO) reduced multiple myeloma cell proliferation in vitro and both intramedullary and extramedullary tumor growth in murine models. Our data show that full-length (FL) ROBO1 is necessary for adhesion of multiple myeloma cells to BM...
stromal and endothelial cells (BMSC and BMEC, respectively) in a heparan sulfate proteoglycan–dependent manner. We found that intravenous injection of mice with ROBO1 KO results in impaired BM homing, with formation of localized bone plasmacytomomas rather than disseminated BM involvement. In add-back experiments, we show that the ROBO1 cytosolic domain is sufficient to rescue ROBO1 KO antiproliferative phenotype, while ROBO1 mutants lacking the cytoplasmic domain, including human-derived G674* truncation, act dominantly negative. We further show that the cytosolic domain of ROBO1 is cleaved in at least three fragments, independently of prior extracellular domain shedding and in the absence of direct SLIT binding, and may localize to the nucleus. Interactomic and RNA-sequencing studies suggest that the ROBO1 C-terminus may have a previously unknown function in RNA processing.

Taken together, these observations warrant further studies to define ROBO1 downstream signaling and ROBO1 C-terminus domain–intrinsic functions.

RESULTS

ROBO1 Is Highly Expressed in Multiple Myeloma Cells and ROBO1 KD Is Cytotoxic in Human Multiple Myeloma Cell Lines

Western blotting and gene expression profiling data show that ROBO1 is highly expressed in human multiple myeloma cell lines and primary cells, in contrast to absent/low expression in normal plasma cells (NPC) and CD138-depleted BM mononuclear cells (BMMC) from healthy donors (Fig. 1A and B; Supplementary Fig. S1A). CD138-depleted BMMCs derived from patients with multiple myeloma (MM-BMMC) express intermediate levels of ROBO1 (Fig. 1B), and ROBO1 expression is increased in precursor condition monoclonal gammapathy of undetermined significance (MGUS) as compared with NPCs (Supplementary Fig. S1B; Supplementary Table S1). Analysis of the CoMMPass database showed variable ROBO1 expression across patients and ROBO1 expression was significantly higher in patients harboring the adverse cytogenetic abnormality t(4;14) (Supplementary Fig. S2A and S2B), as reported previously (15). Similar results were obtained by using the IFA170 gene expression profile database (Supplementary Fig. S2C). Interestingly, MMSET KO in multiple myeloma cell lines harboring a t(4;14) had no significant impact on ROBO1 expression, suggesting that MMSET overexpression is unlikely to be responsible for ROBO1 increased expression in t(4;14) patients (Supplementary Fig. S2D). ROBO1 has been shown to be overexpressed in solid tumors, and its function in the context of solid malignancies has been broadly studied; however, limited data are available regarding ROBO1 expression and function in hematologic malignancies. We first assessed ROBO1 expression across a panel of non–multiple myeloma hematologic cancer cell lines and found that only the diffuse large B-cell lymphoma cell line SU-DHL4 expresses ROBO1 (Fig. 1C; neuroblastoma cell line SH-SYSY and cervical adenocarcinoma cell line HeLa were used as positive controls). Next, we performed shRNA-mediated ROBO1 KD (Fig. 1D) and showed that ROBO1 silencing was cytotoxic across a panel of multiple myeloma cell lines examined (Fig. 1D, shades of red/pink, solid circle symbol; Supplementary Figs. S3 and S4). Conceivably, ROBO1 KD did not elicit cytotoxicity in non–multiple myeloma hematologic cancer cell lines lacking baseline expression of ROBO1 (Fig. 1D, shades of blue, solid square symbol). Intriguingly, ROBO1 KD in ROBO1-expressing SU-DHL4 (Fig. 1D, green line, solid square symbol) as well as SH-SYSY and HeLa (Fig. 1D, shades of yellow, solid triangle symbol) did not affect cell viability, suggesting that mere expression of ROBO1 protein does not reflect a prosurvival function.

Figure 1. ROBO1 is highly expressed in multiple myeloma (MM) and promotes proliferation, while its KD is cytotoxic. A, Western blot analysis showing expression of ROBO1 and SLIT2 across human multiple myeloma cell lines (lanes 1–9), CD138-depleted BMMCs obtained from healthy donors (nBMMC, lanes 10–12) and primary multiple myeloma cells from healthy donors (nBM-PC, lanes 16–17), and primary multiple myeloma cells from multiple myeloma patient (MM, lane 18). Red rectangles outline multiple myeloma–derived samples. 2B3T cells were used as positive control (lane 19). GAPDH was used as loading control. Multiple myeloma cell lines harboring t(4;14) are outlined in red. B, ROBO1 gene expression data from IFAM170 dataset comparing multiple myeloma, CD138-depleted BMMCs derived from patients with multiple myeloma (MM-BMMC), and NPCs from healthy individuals. The middle line represents median value; the boxes includes values between the lower and upper quartile. Kruskal–Wallis one-way analysis was used to test sample distribution, with Dunn multiple comparison test. ***, P < 0.001. C, Western blot showing ROBO1 expression across a panel of human multiple myeloma (lanes 1–7); B-cell lymphoma (lanes 8–13); T-cell acute lymphoblastic leukemia (T-ALL lane 14), or myeloid leukemia (AML, lanes 15–16) cell lines. Neuroblastoma cell line SH-SYSY (lane 17) and cervical adenocarcinoma cell line HeLa (lane 18) were used as positive controls for high ROBO1–expressing malignancies. GAPDH was used as loading control. D, ROBO1 silencing was performed via shRNA in a panel of multiple myeloma cell lines (shades of red/pink, solid circle symbol), non–multiple myeloma hematologic cancer cell lines (shades of blue, *; except high ROBO1-expressing SU-DHL4 in green, *), as well as SH-SYSY and HeLa cells (shades of yellow, *). Apoptosis was assessed via flow cytometry with annexin V/propidium iodide staining at 3, 5, and 7 days after puromycin selection. For each time point, graph shows percentage of viable cells (annexin V/propidium iodide double negative) in comparison with cells transfected with scrambled shRNA. E, Western blot analysis showing three ROBO1 KO OPM2 clones generated with three distinct sgRNAs (Sg0, Sg2, and Sg3). SLIT expression was unchanged in ROBO1 KO clones. β-tubulin served as a loading control. F, Schema of FL and mutant truncated ROBO1 add-backs. Δcyt ROBO1 mutant lacks the cytosolic domain, while MP–Δcyt ROBO1 mutant lacks the extracellular domain and is anchored to the cell membrane via a myristoylated-palmitoylated anchor (MyrPalm). All add-back constructs were tagged with a C-terminus triple FLAG tag. FN3, fibronectin type III domain; fgc, Ig-like C2 domain. G, Western blot showing expression of add-back constructs in OPM2 KO cells in relationship to WT OPM2 cells (first lane). A ROBO1 antibody recognizing the C-terminus (ROBO1 C-term) was used in top panel, while an antibody recognizing the N-terminus was used in the second panel (ROBO1 N-term). An antibody recognizing FLAG was used in the third panel. GAPDH was used as a loading control. Asterisk outlines a nonspecific band. Molecular weights are marked on the right. H, Histogram bars represent fold proliferation compared with day 0 (D0; day of seeding) of ROBO1 WT (solid black column), KO (striped gray column), and FL add-back OPM2 monocytes (solid shades of gray columns) during 4 days in culture in a 3D encapsulation system. *, P ≤ 0.05; ***, P ≤ 0.001, as calculated via t test for WT versus KO and KO versus FL. Experiments were performed in quadruplicate, with two biological repeats. I, Histogram bars represent fold proliferation compared with D0 of WT KMS11 (solid black column) cells and two distinct ROBO1 KO (striped gray column) and respective FL add-back (solid shade of gray columns) KMS11 monocytes during 4 days in culture in a 3D encapsulation system. *, P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001, as calculated via t test for WT versus KO and KO versus FL. Experiments were performed in quadruplicate with two biological repeats.
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A

B

C

D

E

F

G

H

I
To study protein structure and function and to establish long-term ROBO1-null clones, we performed CRISPR-Cas9–based ROBO1 gene KO in MM.1S, MOLP8, NCI-H929, KMS11, and OPM2 multiple myeloma cell lines using three distinct single-guide RNA (sgRNA; Sg0, 2, and 3). We were able to establish three biallelic ROBO1 KO clones in OPM2, one per each guide (Fig. 1E), as well as several Sg2- and Sg3-based ROBO1 KO clones in KMS11 (Supplementary Fig. S5A). Either the other cell lines did not grow monoclones (MM.1S and MOLP8) or the monoclones were all ROBO1 WT (H929, Supplementary Fig. S5B). We believe we were able to produce biallelic ROBO1 KO monoclones in OPM2 and KMS11 due to their increased plasticity and tolerance to perturbations in prosurvival genes, compared with other multiple myeloma cell lines. Importantly, ROBO1 KO had no impact on SLIT2 expression (Fig. 1E).

To study the phenotypic consequences and cause–effect relationships between ROBO1 loss and cellular phenotypes, we transduced ROBO1 KO OPM2 clones with lentiviral vectors expressing FL ROBO1 cDNA, ROBO1 mutant devoid of the intracellular domain (ΔCyts), or ROBO1 mutant expressing the cytosolic domain only with a myristoylated-palmitylated anchor motif to guide cellular membrane localization (MP-Cyt, Fig. 1F). As polyclonal cells transduced with FL ROBO1 had a ROBO1 expression that was significantly lower than endogenous ROBO1, we performed single-cell sorting to identify monoclones with highest ROBO1 expression (Supplementary Fig. S6A and S6B). The FL polyclonal cell population was used for in vitro studies. Distinct FL add-back monoclones were used for in vitro studies (Supplementary Fig. S6A and S6B, outlined in red). Monoclonal FL1 is referred in the manuscript as FL ROBO1 add-back constructs were tagged at the C-terminus with a triple FLAG tag and demonstrated the expected molecular weights of 180, 102, and 85 kDa, respectively, upon Western blotting (Fig. 1G). FL ROBO1 add-backs were also established in four distinct ROBO1 KO KMS11 clones (Supplementary Fig. S7).

**ROBO1 KO Decreases Multiple Myeloma Cell Proliferation In Vitro and Inhibits Intramedullary and Extramedullary Tumor Growth in Murine Models**

On the basis of shRNA experiments, we hypothesized that ROBO1 has a prosurvival function in multiple myeloma. Standard 2D viability/proliferation assays failed to show any biologically significant difference between ROBO1 WT versus KO versus FL, and we hypothesized that activation of ROBO1 may be dependent on the presence of a 3D microenvironment. Therefore, we performed encapsulation of ROBO1 WT, KO, and FL ROBO1 add-back OPM2 and KMS11 multiple myeloma cells in a hydrogel matrix, which allows for study of multiple myeloma cell growth in a 3D system (16). ROBO1 KO significantly decreased multiple myeloma cells proliferation compared with WT and adding back FL ROBO1 partially (OPM2) or fully rescued (KMS11) this phenotype, demonstrating that ROBO1 is a necessary factor for multiple myeloma proliferation (Fig. 1H and I). To test the impact of ROBO1 KO in vivo, we initially used a plasmacytoma model of extramedullary multiple myeloma growth where ROBO1 KO or WT cells are injected subcutaneously in the flank of recipient, NOD SCID gamma (NSG) mice. PET-CT scans showed a statistically significant higher tumor volume and FDG avidity in ROBO1 WT versus ROBO1 KO plasmacytoma (Fig. 2A and B). Because multiple myeloma is characterized by intramedullary growth and dissemination, we next examined the effect of ROBO1 KO on tumor growth within the BM niche using the μ-SCID mouse model, in which multiple myeloma cells are injected directly into the BM cavity of femora from syngeneic donor mice. These multiple myeloma–containing femora are then implanted subcutaneously in the flank of recipient mice, allowing for subsequent monitoring of tumor cell growth in the context of the murine BM microenvironment (Fig. 2C, schema; ref. 17). The μ-SCID model revealed an even more profound inhibitory effect of ROBO1 KO on cell growth as compared with the plasmacytoma model (Fig. 2D and E). Interestingly, we observed complete failure to engraft (no tumor growth) in 5 of 15 ROBO1 KO grafts as compared with only 1 of 15 for WT (Fig. 2D, Supplementary Fig. S8). These data suggest that ROBO1 KO may compromise the initial phases of multiple myeloma–BM niche interaction necessary for tumor engraftment.

**ROBO1 Activation Triggers Cytoskeleton Rearrangement and Mediates Multiple Myeloma Adhesion to BMEC and BMSC**

To determine whether ROBO1 is necessary for multiple myeloma–BM niche interaction, we performed adhesion assays of WT versus KO ROBO1 OPM2 and FL ROBO1 add-back.
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A. WT vs KO

B. Tumor volume and Tumor SUVmax

C. Robo1 WT or KO

D. BM engraftment failure

E. Tumor volume

F. Fold change in adhesion

G. Fold change in adhesion compared to WT

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Our data show that ROBO1 KO significantly decreased adhesion to BMEC lines and MM-BMSCs derived from patients (MM-BMSC#2 and #3; Fig. 2F), while adding back FL ROBO1 completely rescued this defective phenotype (Fig. 2G). Similar data were obtained when using KMS11 cells (Supplementary Fig. S9). We next showed that ROBO1-mediated BMEC adhesion was dependent on heparan-sulfate proteoglycans (HSPG), because pretreatment with heparin sulfate decreased adhesion of WT and ROBO1 FL to BMEC at a level comparable with ROBO1 KO cells (Fig. 2G). These data are consistent with previous reports that HSPGs stabilize ROBO1/SLIT2 interaction, which appears critical for downstream signaling (18). Next, to assess the consequences of ROBO1 activation on cytoskeleton rearrangement, we treated MM.1S cells with exogenous SLIT2 for 15 minutes. By using immunofluorescence staining, we show that SLIT2 triggers formation of lamellipodia, suggesting a role for this pathway in cell migration (Fig. 3A). To corroborate these data and assess for a causative role of ROBO1 in cytoskeleton rearrangement, we performed a similar experiment in OPM2 ROBO1 KO or FL and FL F add-backs (Fig. 3B). Immunofluorescence staining showed lack of cytoskeleton rearrangement in baseline conditions in both ROBO1 KO and the two FL F add-back clones. However, upon SLIT2 administration, FL ROBO1 clones displayed cytoskeleton activation and formation of lamellipodia in contrast to ROBO1 KO cells. Importantly, immunofluorescence images suggested ROBO1 nuclear localization and localization in the lamellipodia upon treatment with SLIT2.

**ROBO1 KO Leads to Impaired BM Homing and Dissemination In Vivo, with Predominant Formation of Bone Plasmacytoma**

To further study the role of ROBO1 in multiple myeloma dissemination and homing in vivo, we used a disseminated mouse model where multiple myeloma cells are injected intravenously into the tail vein of NSG mice (17). The characteristic phenotype is diffuse homing of multiple myeloma cells to the intramedullary space (typically femora and spine), where they proliferate and cause hindlimb paralysis presumably to pathologic fractures and/or cord compression (Fig. 3C, schema). Mice injected with ROBO1 FL cells recapitulated this expected pattern of disseminated disease, with 100% of mice developing hindlimb paralysis in the context of widespread BM invasion. In contrast, 100% of mice injected with ROBO1 KO cells developed hindlimb and/or forelimb plasmacytomas that led to impaired mobility and inability to properly feed due to rapid growth of bulky tumor (Fig. 3D). As a consequence of meeting humane endpoint, these animals were sacrificed in the absence of signs of paralysis and we did not observe any statistically significant difference in overall survival (OS) between ROBO1 KO and FL mice (Supplementary Fig. S10). The mean time to sacrifice was 87 days for FL mice (75–125) and 108 days for KO mice (76–168). IHC analysis of femora harvested from mice injected with ROBO1 FL cells showed extensive infiltration with multiple myeloma cells, in stark contrast to the minimal or absent BM plasmacytosis detected in femora from mice injected with ROBO1 KO multiple myeloma cells (Fig. 3E). The pattern of disease observed in our ROBO1 KO animals is analogous to development of plasmacytomas associated with minimal or no BM involvement observed in patients (19). In contrast to overt multiple myeloma, these disorders are often amenable for localized treatment with either radiotherapy or surgery, with an intent to cure (20). We next assessed ROBO1 expression via IHC in 10 biopsy samples from patients with solitary plasmacytoma (SPC) and compared it to 14 BM biopsy samples from patients with multiple myeloma. We detected expression of ROBO1 only in 1 SPC (dim expression) as opposed to 14 out of 14 BM biopsy specimens (11 bright, 3 dim; Fig. 3F; P < 0.0001). We were also able to identify 4 matched samples of patients who had biopsy of both BM and a plasmacytoma site within a few weeks from each other. We found that ROBO1 expression was lost in the plasmacytoma as compared with the BM-resident plasma cells (Fig. 3G).

**ROBO1 Cytosolic Domain Is Cleaved into Multiple Fragments Independently of Direct SLIT2 Binding and of Extracellular or Juxtacellular Domain Expression**

To understand the molecular mechanisms mediating the observed proproliferative and BM-homing phenotypes, we first performed FLAG immunoprecipitation (IP), followed by Western blot analysis for known ROBO1 interacting partners in ROBO1 FL OPM2. KO cells were used as control. We detected specific ABL binding (Fig. 4A). Interestingly, across multiple pull-down experiments, we observed a consistent pattern of banding in cells transduced with FL- and
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A

Baseline
SLIT2

ROBO1

β-Actin

B

FLAG Nucleus β-Actin Overlap

ROBO1 KO baseline
ROBO1 KO PLUS SLIT2
FL ROBO1 baseline
FL ROBO1 PLUS SLIT2
FL ROBO1 PLUS SLIT2

C

Intravenous tail vein injection

Survival analysis
IHC on target organs
BM homing and growth

ROBO1 KO or FL MM cells

D

Forelimb
Hindlimb

KO #0
KO #1
KO #3

E

FL
H&E CD138

KO
H&E CD138

4× 20× 4× 20×

4× 20× 4× 20×

F

MM

#1 #2 #3 #4

#5 #6 #7 #8

SPC

G

MM

#1 #2 #3 #4

PC

#1 #2 #3 #4

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Cyt ROBO1, but not in cells transduced with ΔCyto ROBO1, and only when a ROBO1 antibody recognizing the C-terminus, but not one recognizing the N-terminus, was used for detection (Fig. 4B, left and right panels, respectively). A similar pattern of banding was observed in 293T cells transfected with an FL ROBO1 construct tagged with a C-terminal LAP tag, suggesting this was not an artifact related to either cell line or FLAG tagging, but rather represented cleaved ROBO1 fragments (Supplementary Fig. S11A). Endogenous ROBO1 IP was technically difficult with low signal-to-noise ratio. However, we were able to identify a putative cleaved fragment (C.F.) also in this setting (Supplementary Fig. S11B). To confirm the identity of these bands, we transiently transfected 293T cells with MP-Cyt ROBO1 or an empty control vector, and then performed FLAG IP, followed by Coomassie staining of the gel. As shown in Fig. 4C, the gel was divided into four distinct sections: section one contains the entire MP-Cyt ROBO1 band (asterisk), while sections 2, 3, and 4 each contained only one of the lower molecular weight bands detected in coimmunoprecipitation (arrows). LC/MS identified ROBO1 peptides in all four sections, consistent with these bands representing cleavage of the C-terminus of the cytosolic domain of ROBO1. The peptide sequence detected by LC/MS in section 2 (SECT2), containing the highest molecular weight cleaved band, is schematically represented in relationship to FL ROBO1 and MP-Cyt ROBO1 protein sequences (Fig. 4D). Outlined in yellow is the amino acid sequence containing the putative cleavage site between amino acid 883 and 1061. In contrast to a prior report, our data show that in multiple myeloma, ROBO1 cleavage occurs efficiently also in cells expressing only MP-Cyt ROBO1, ruling out the need for direct SLIT2 binding and extracellular domain cleavage as upstream events of cytosolic cleavage (21). To ascertain whether membrane localization of ROBO1 is necessary for C-terminus cleavage, we transiently expressed a mutant ROBO1 (Cyt ROBO1) composed only of the intracytosolic ROBO1 domain without a Myr-Palm, membrane-anchoring motif. MP-Cyt and ΔCyt ROBO1 were used as positive and negative control, respectively. Our data show that Cyt ROBO1 is cleaved according to same pattern as MP-Cyt ROBO1, although the stoichiometry of parental Cyt ROBO1 versus smaller fragments suggests that cleavage occurs in a less efficient manner in the absence of membrane localization (Fig. 4E). We next asked whether cleaved ROBO1 may translocate to the nucleus as suggested by our prior immunofluorescence staining (Fig. 3B). Immunofluorescence staining of OPM2 cells expressing MP-Cyt ROBO1 shows both cell membrane (yellow arrow) and nuclear localization of ROBO1 as compared to ROBO1 KO cells (Fig. 4F). As TP53 has been previously implicated in regulating genes highly expressed in t(4;14) multiple myeloma, we assessed ROBO1 expression in two distinct p53 KO OPM2 clones (15). While we did not observe any significant difference in the level of ROBO1, a previously not detected band of the approximate molecular weight of 75 to 100 KDa was evident in both p53 KO monoclonal clones (Fig. 4G). This band could potentially represent ROBO1 C.F., suggesting that in the absence of TP53, ROBO1 cleavage is increased.

**ROBO1 Cytosolic Domain Is Necessary and Sufficient to Mediate Proproliferative Functions in Multiple Myeloma**

To investigate the function of ROBO1 cytosolic domain, we performed FLAG IP, followed by Coomassie staining and LC/MS, in two distinct ROBO1 KO clones (Sg2 and Sg3) and three distinct FL ROBO1 add-back clones (FL, F, and W) OPM2. Pathway analysis identified with a high degree of confidence proteins participating in DNA repair (PRKDC), actin-cytoskeleton remodeling (TLN1, ENO1, IQGAP1, and ARHGEP2) and proteostasis (HYOU1 and EDEM3; Fig. 5A). We also performed a similar pull-down experiment followed by LC/MS in 293T cells transiently expressing MP-Cyt ROBO1 versus empty vector to specifically assess interacting partners of the cytosolic domain of ROBO1. In this context, the mRNA binding protein and posttranslational regulator MS1 emerged as a novel, putative interacting partner of ROBO1 (Supplementary Fig. S12, arrow). In-depth analysis of the ROBO1 interactome showed significant binding to factors involved in RNA processing and metabolism (Fig. 5B, arrows). While a role for ROBO1 in cytoskeleton rearrangement and migration

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**Figure 4.** The ROBO1 C-terminus domain is cleaved in multiple fragments in the absence of a SLIT2 binding domain or transmembrane domain. 
- **A.** First four lanes show FLAG IP in ROBO1 KO and ROBO1 FL OPM2 cells with or without exogenous stimulation with SLIT2N (S2N), followed by Western blotting to detect ABL. Last four lanes show whole-cell lysate protein input in the same samples. GAPDH served as a loading control. **B.** Left, FLAG IP in ROBO1 WT, KO, FL, ΔCyt, and MP-Cyt OPM2, followed by Western blotting with an antibody recognizing the ROBO1 C-terminus. FL ROBO1 is recognized as the high molecular weight band in the FL IP lane. Asterisk identifies the MP-Cyt ROBO1 band. Arrows point at three distinct bands detected only in FL and MP-Cyt ROBO1 IP. Right, coimmunoprecipitation with same FLAG IP samples, followed by Western blotting with an antibody recognizing the ROBO1 N-terminus. An overexposed image is presented to show absence of bands previously detected with ROBO1 C-terminus antibody. **C.** FLAG IP in 293T cells transiently transfected with an empty vector (BB) or a vector expressing MP-Cyt ROBO1 (MP-CYT), followed by SDS page and Coomassie blue staining. Gel was subsequently divided in sections 1 to 4, as represented in the figure. Each section contains a band present in MP-Cyt ROBO1 IP, but not control. Asterisk identifies the MP-Cyt ROBO1 band. Gel sections were subsequently submitted for LC/MS. **D.** Alignment of cytosolic domain of FL ROBO1 (transmembrane domain is bolded and underlined) with MP-Cyt ROBO1 (myristoylated-palmitoylated anchor motif is bolded) and the most N-terminus ROBO1 peptides detected in section 2 (SECT2) by LC/MS, respectively submitted for LC/MS.

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Figure 5. The ROBO1 cytosolic domain mediates ligand-independent, proproliferative functions. A, Protein extract from OPM2 Sg2 and Sg3 ROBO1 KO and FL, FL F, and FL W monoclonal add-backs were subjected to FLAG IP, followed by LC/MS. The volcano plot shows the top differentially represented proteins in the add-back pulldowns as compared with KO (false discovery rate < 0.1, log2 fold change > 1.5). B, Protein extract from OPM2 ROBO1 KO or stably expressing FL ROBO1 were subjected to FLAG IP, followed by LC/MS. The figure is a treemap, hierarchical representations of the specific ROBO1 interacting partners based on R pathway analysis. The size of each square is proportional to the number of proteins present in the sample that enriched the pathway. The color is based on the false discovery rate (only pathways with FDR < 0.05 are shown). Red arrow points to pathways involved in RNA metabolism. TC-NER, transcription-coupled nucleotide excision repair. C, Histogram bars show fold change in proliferation compared to day 0 (D0) for ROBO1 WT, KO, FL, ΔCyt, and MP-Cyt OPM2 cultured for 4 days in a 3D encapsulation system. **, P ≤ 0.01; ***, P ≤ 0.001.
was expected on the basis of published data, the interaction with the DNA repair factor PRKDC and with proteins involved in RNA processing was unexpected and suggested a potential novel role for ROBO1 cytosolic domain. We therefore next asked if the cytosolic domain of ROBO1 alone could be sufficient to mediate the proproliferative function of FL-ROBO1. We thus assessed proliferation of WT, KO, FL, ΔCyt, and MP-Cyt ROBO1 OPM2 clones in our 3D system. Our data show that the MP-Cyt ROBO1 add-back fully rescues the proliferation defect of ROBO1 KO cells, while ΔCyt ROBO1 does not (Fig. 5C). Thus, the ROBO1 cytosolic domain is sufficient to drive multiple myeloma proliferation.

As an orthogonal method to investigate ROBO1 function in multiple myeloma, we performed RNA-sequencing analysis of ROBO1 WT versus KO plasmacytoma from the previously described plasmacytoma mouse model (Fig. 2A and B). As expected, ROBO1 was the most differentially expressed gene (Supplementary Fig. S13A and S13B). When looking at the top five most differentially expressed genes, THBS2, CPM1, and FBLN2 emerged as significantly highly expressed in ROBO1 WT versus control. These genes encode for proteins participating in cell-to-cell interaction and extracellular membrane composition. Gene-set enrichment analysis confirmed significant overrepresentation of epithelial-to-mesenchymal transition genes in ROBO1 WT tumors (Supplementary Fig. S14). High-stringency reactome analysis showed high expression of genes involved in ROBO1/SLIT2 pathway and extracellular matrix composition and cell–cell contact (Fig. 6A, green and red arrows, respectively). Consistent with our interactomic analysis, genes involved in RNA processing were also significantly overexpressed in ROBO1 WT compared with KO cells (Fig. 6A, blue arrows), suggesting a previously unknown function of ROBO1 in direct binding and potential regulation of RNA processing.

**ROBO1 Truncating Mutations Devoid of Intracellular Domain Act as Dominant Negative**

ROBO1 somatic mutations have been recently detected in primary multiple myeloma cells from patients (13, 22). In particular, three of the five monoallelic mutations initially described are expected to result in a truncated protein (S565*, G674*, K863fs*) encompassing only the extracellular domain of ROBO1 (Supplementary Fig. S15A). The functional relevance of these mutations was unknown at the time of this study. However, RNA-sequencing data were available for the G674* mutant, and we set out to test whether this mutation results in expression of a truncated protein. Western blot analysis showed that when transfected in ROBO1 WT or KO OPM2 cells, G674* mutant cDNA results in the expression of a protein of expected molecular weight (~70 kDa; Fig. 6B). As this was a monoallelic mutation, we decided to study the functional relevance of G674* expression against WT background. To this end, we expressed ΔCyt ROBO1, MP-Cyt ROBO1, G674* ROBO1, or an empty vector in ROBO1 WT OPM2 cells and then assessed proliferation using our 3D system. Expression of MP-Cyt ROBO1 did not affect OPM2 proliferation; however, both ΔCyt ROBO1 and G674* ROBO1 significantly inhibited ROBO1 WT cell proliferation, consistent with a dominant-negative effect (Fig. 6C).

**DISCUSSION**

The BM niche plays a key role in the pathogenesis of multiple myeloma and other hematologic malignancies by supporting tumor cell proliferation, dissemination, and resistance to therapy (3, 4). There is a growing interest in identifying receptors involved in pathogenic multiple myeloma–BM niche interaction to design specific, molecularly targeted therapies. In this study, we assessed the role of the transmembrane receptor ROBO1 in multiple myeloma pathogenesis in the context of multiple myeloma–BM niche interaction. We discovered that ROBO1 is necessary for adhesion of multiple myeloma to BMSC and BMEC and dissemination and homing of circulating multiple myeloma cells to BM sites. Unexpectedly, we identified a direct proproliferative and prosurvival function of ROBO1 in multiple myeloma that is dependent on the sole presence of its cytosolic domain, suggesting a dual function for ROBO1 in multiple myeloma pathogenesis (Fig. 6D). While no ROBO1 dependency was identified in multiple myeloma cell lines by screening tools (https://depmap.org/portal/), through more sensitive tools, our work suggests that ROBO1 has a proproliferative function in multiple myeloma. We demonstrate for the first time that the ROBO1 C-terminus is cleaved in at least three distinct cytosolic fragments in a process that does not require direct SLIT2 binding, prior extracellular domain shedding, or membrane anchoring. This is in contrast with a prior report in liver cancer cell lines where ectodomain cleavage was reported as a necessary step ahead of C-terminus cleavage (21, 23). The identity of the protease responsible for ROBO1 C-terminus cleavage remains unknown. This is the subject of ongoing studies and may represent a suitable molecular target to block ROBO1 proproliferative function. Importantly, our data show that isogenic OPM2 cells null for p53 may have increased ROBO1 processing, suggesting that TP53 may function as a gatekeeper of ROBO1 cleavage and that the role of ROBO1 cleavage in mediating the ominous prognosis of del(17) multiple myeloma may be worth exploring.

Interestingly, we cloned and expressed in ROBO1 WT and KO cells a human-derived ROBO1 truncation whose expression and phenotypic outcome were unknown (13). We show that the G674* mutant results in an expressed protein that has a dominant-negative phenotype based on proliferation assay. This mutation was found to be expressed at a very low level and against a background of low WT ROBO1 expression in the index patient, suggesting lack of significant biological impact on the pathway (24). Follow-up studies suggested that mutations in the ROBO1 genes may be passenger mutations and simply related to the large size of the ROBO1 gene. In fact, our functional studies confirm this speculation and reconcile the seemingly paradoxical lack of expression of G674* ROBO1 mutant allele based on its detrimental function in multiple myeloma growth.

Somatic loss-of-function mutations in ROBO1 and ROBO2 have been reported in paired samples of patients with myelodysplastic syndrome progressing to acute myeloid leukemia, and overexpression of ROBO1 and ROBO2 in leukemia cell lines caused decreased proliferation and apoptosis, consistent with a tumor suppressor function (25). On the basis of our data, however, in multiple myeloma, ROBO1 serves as
Figure 6. Putative role of ROBO1 in RNA metabolism and model of dual function in multiple myeloma (MM). A, Reactome analysis of RNA sequencing from ROBO1 WT versus KO plasmacytoma (three per group). FDR was set at < 0.005. Bars on the right are enriched in ROBO1 WT, while bars on the left are enriched in ROBO1 KO. Red arrows point at pathways involved in extracellular matrix signaling, green arrows point at pathways involved in SLIT/ROBO1 signaling, and blue arrows point at pathways involved in RNA metabolism. B, OPM2 ROBO1 WT cells were transduced with an empty lentiviral vector (BB) or a lentivirus expressing G674* ROBO1. Western blot analysis with ROBO1 antibody recognizing N-terminus domain shows that G674* ROBO1 is expressed. GAPDH is used as loading control. C, Histogram bars show fold change in proliferation compared with day 0 (D0) for ROBO1 WT OPM2 cells transduced with an empty lentiviral vector (BB) or a vector-expressing ΔCyt, MP-Cyt, or G674* ROBO1 and cultured for 4 days in a 3D encapsulation system. **, P ≤ 0.01. D, Schema of model of ROBO1 dual function in multiple myeloma. As a full transmembrane receptor, ROBO1 mediates multiple myeloma adhesion and dissemination to the BM in a ligand-dependent manner (left). Upon cleavage by an as yet unidentified protease (scissors), the cytosolic domain of ROBO1 may translocate to the nucleus and potentially participate in RNA metabolism or regulation, resulting in a ligand-independent proproliferative and prosurvival function (right). RBC, red blood cell.
a proto-oncogene, eliciting multiple myeloma proliferation and survival and mediating interaction with key elements in the BM niche. Taken together, our results identify and characterize for the first time a pathogenetic role for ROBO1 in multiple myeloma and set the bases for future investigations regarding the identity of the ROBO1 protease, the impact of ROBO1 overexpression in immunocompetent multiple myeloma mouse models such as the Vk-Myc mouse, and confirmation of ROBO1 interacting partners and the role of ROBO1 in RNA metabolism (26).

**METHODS**

**Cell Lines and Cell Culture**

Human multiple myeloma cell lines were available in our laboratory or purchased from ATCC and maintained as described previously (16). The human BM endothelial cell line BMEC-60 was kindly provided by Dr. CE van der Schoot (CLB, Amsterdam, The Netherlands) and was cultured in endothelial culture medium (EGM-2, Lonza) with full supplements. The human BM endothelial cell line TrHBMEC was a kind gift of Dr. Babette B. Weksler (Weill Medical College of Cornell University, New York, NY) and was cultured in DMEM 0.45% glucose-glutamax, 5% FBS, 10 mmol/L HEPES, 100 U/mL penicillin, and 100 μg/mL streptomycin in cell culture flasks precoated with 0.2% sterile gelatin (in distilled water). All other cell lines used in this study were purchased from ATCC and maintained as per instructions. All cell lines were maintained in sterile conditions in a cell culture incubator at 37°C with 5% CO2. All experiments were conducted within 1 to 3 weeks or 3 to 5 passages from cell thawing. Cell lines were tested to rule out Mycoplasma contamination prior to experiments and before freezing by using the MycoAlert Mycoplasma Detection Kit (Lonza). All cell lines used in this article were authenticated via short tandem repeat profiling prior to use.

**Primary Multiple Myeloma, NPCs, and BMSCs**

BM aspirate samples were obtained from patients diagnosed with multiple myeloma, after obtaining informed consent and approval by the Institutional Review Board of the Dana-Farber Cancer Institute (Boston, MA). BMMCs were separated by Ficoll-Paque PLUS (GE Healthcare), and multiple myeloma cells were enriched by CD138-positive selection with magnetic-activated cell separation microbeads (Miltenyi Biotec) prior to snap freezing. BMSCs were established by culturing CD138-negative BMMs for 4 to 6 weeks in DMEM containing 100 U/mL penicillin and 100 μg/mL streptomycin, supplemented with 20% (v/v) FBS. Normal BMSCs were obtained by processing discarded filters from healthy BM donor in a similar fashion, after obtaining informed consent and approval by the Institutional Review Board of the Dana-Farber Cancer Institute. NPCs obtained via CD138-positive selection with magnetic-activated cell separation microbeads (Miltenyi Biotec) were snap frozen for future use.

**shRNA-Mediated ROBO1 Silencing**

shRNA constructs for ROBO1 silencing were ordered from Dharmacon, Thermo Scientific [the RNAi Consortium (TRC) Lentiviral shRNA]. Mature antisense sequences can be found in Supplementary Methods. shRNA4 was chosen for initial experiments based on best KD efficiency. shRNAs was subsequently used to confirm data. Lentiviral transduction was performed as described previously (16). Cells were selected in puromycin-containing media for up to 7 days. ROBO1 KD was confirmed using Western blotting of cells harvested 3 days after puromycin. Aliquots of cells were collected 3, 5, and 7 days after puromycin selection to assess for apoptosis via flow cytometry for annexin V/propidium iodide staining (PI) as described in Supplementary Methods. Scrambled-transfected cells were used as controls for procedure-related toxicity.

**ROBO1 and SLIT2 Constructs**

Human ROBO1 (MHS6278-213246291, clone ID 9054509, Accession BC171855) and SLIT2 (MHS6278–211691098, clone ID 40125741, Accession BC171910) cDNA were purchased from GE Healthcare. Details regarding generation of mutants and tagged constructs can be found in Supplementary Materials.

**LC/MS**

SDS page gels were stained with QC Colloidal Coomassie (Bio-Rad) according to manufacturer protocol. Gel was cut in sections and submitted for LC/MS at the Taplin Mass Spectrometry Facility at Harvard Medical School.

**FLAG, LAP and ROBO1 Immunoprecipitation**

Cell pellets were lysed in high ionic strength (400 mmol/L NaCl) lysis buffer (25 mmol/L Tris-HCl, pH 7.6, glycerol 2.5%, Triton X-100 1%, and MgCl2 500 μmol/L) with protease and phosphatase inhibitors. Protein concentrations were quantified via Bradford assay. An equal amount of protein sample was immunoprecipitated via incubation with anti-FLAG flag A4596, Millipore-Sigma), loaded on Pierce Micro-spin Columns (88879, Thermo Fisher Scientific) and washed three times with lysis buffer. 3X FLAG peptide was used to elute IP from matrix. For LAP IP, an equal amount of protein sample was immunoprecipitated via GFP-Trap Magnetic Agarose (GTMA-10, Chromotek) according to Pierce Classic Magnetic IP/Co-IP Kit (88804, Thermo Fisher Scientific) instructions. After adding 4X LBS buffer, samples were boiled at 90°C for 10 minutes and subjected to SDS page as above for downstream Western blotting or LC/MS. For ROBO1 endogenous protein IP, we used the Pierce Magnetic IP Kit according to manufacturer’s instructions (Thermo Fisher Scientific).

**Multiple Myeloma Cell Adhesion Assay**

Primary BMSCs and human endothelial cell lines BMEC60 and TrHBMEC were plated at a density of 1 × 104 per well in a 96-well plate 1 day before the adhesion assay. Adhesion assay was performed as described previously (16). Brieﬂy, BMEC lines (BMEC60 and TrHBMEC) or MM-BMSCs were allowed to adhere overnight in 96-well plates. The following day, an equal amount of calcine AM–stained cells were seeded in triplicates and allowed to adhere for 90 minutes, followed by washing. The number of adherent cells was calculated based on the fluorescent intensity as assessed via spectrophotometer and normalized against initial seeding density. These values were then normalized to ROBO1 WT.

**CRISPR-Cas9 KO of ROBO1, TP53, and MMS2 (NSD2)**

We designed sgRNA targeting hsROBO1 by using the MIT CRISPR tool (crispr.mit.edu). Sequences are as follows: sgRNA0: TCTTAC GTATGATGACG; sgRNA2: TACACCCTGAAAGTAGGCGC; and sgRNA3: ACCCTGTACCTACTAGTCAGGA. ROBO1 sgRNA was then cloned into pSpCas9(9BB)-2A-GFP vector as reported previously (27). Multiple myeloma cell lines and 293T cells were transiently transfected via electroporation (multiple myeloma cells) or DNA/PEI (polyethylenimine) complex, as described previously, and FACs sorted, with one cell per well into 96-well plates, gating for GFP positivity (transfected) and DAPI/PI negativity (viable; ref. 16). Monoclonal lines were expanded and screened via Western blot. Identified KO monoclonal cell lines were genotyped; primer sequences for genomic PCR can be found in Supplementary Data. pSpCas9(9BB)-2A-GFP (PX458) was a gift from Feng Zhang (Addgene plasmid, #48138).
TP53 and MMSET KO was performed according to a modified ribonucleoprotein (RNP) protocol (see Supplementary Material; M. Moscioni and colleagues, manuscript in preparation; ref. 28). CRISPR RNA (crRNA) ATTO550-labeled tracrRNA and recombinant Cas9 were obtained from Integrated DNA Technologies (IDT). CrRNA sequences were as follows: TP53 crRNA AA: CCATTGTCATATCGTCCG; TP53 crRNA AC: TCCACTCGGATAGATCGCT; MMSET crRNA AA: CCAAAAGTGTCGGGTTACCCT; and MMSET crRNA AE: ACACAAG AGCTGCTTGAGCA. A nontargeting crRNA was used as negative control. We used two distinct TP53 KO OPM2 monoclones and bulk MMSET KO H92, OPM2, and KMS11 cells.

Murine Xenograft Models

Five-week-old female NSG mice were used for all studies (Charles River). All animal studies were performed under protocols approved by the Animal Ethics Committee of the Dana-Farber Cancer Institute. For the plasmacytoma model, mice were subcutaneously injected, bilaterally with 5 × 10^6 viable OPM2 ROBO1 WT or KO cells in a 1:1 ratio with Matrigel (Corning). Eight mice per group were used. For the μ-SCID model, donor mice were anesthetized with isoflurane and bilateral femora harvested in a sterile fashion. The femoral head was then cut and 10 μL of BM aspirated and discarded. OPM2 ROBO1 WT or KO cells were then injected intramedullary at a concentration of 1 × 10^6 cells in 10 μL sterile HBSS. The femoral head was then sealed with Matrigel prior to subcutaneous implantation into recipient mice. Eight mice per group were implanted with two implants each (one on each flank) except for one mouse in each group that received only one implant. For both models, tumors were allowed to grow. Mice were imaged with PET-CT as below, once tumor in the WT cohort reached 2 cm in length or 2 cm^3 volume or if mice appeared moribund, to prevent unnecessary morbidity. Details on PET-CT protocol can be found in Supplementary Data.

For the disseminated mouse model, mice were injected in the right lateral tail vein with 500,000 OPM2 ROBO1 KO or FL cells resuspended in 100 μL HBSS media. Five mice per group were used. Mice were monitored for development of plasmacytoma or paralysis. Mice were sacrificed once endpoint was reached. Tumors and bilateral femora were harvested, fixed in 10% formalin overnight, and then preserved in 70% ethanol until processing for IHC with hematoxylin and eosin or with CD138 (#12922, Cell Signaling Technology) or ROBO1 antibodies.

Statistical Analysis

Experiments were performed in three biological replicates, unless otherwise specified. Biological triplicates were used unless otherwise specified. Statistical significance was determined by Student t test when comparing two populations or ANOVA when comparing more than two populations (ns: P > 0.05; * P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001; ****, P ≤ 0.0001).

Authors’ Disclosures

G. Bianchi reports grants from Damon Runyon Cancer Research Foundation, American Society of Hematology, and International Myeloma Foundation during the conduct of the study, as well as personal fees from Pfizer, Karyopharm, and MJH outside the submitted work. A.M. Roccaro reports grants from Italian Association for Cancer Research (AIRC-IG248689) and Fondazione Regionale per la Ricerca Biomedica (Transcan-2-ERANET) during the conduct of the study, as well as personal fees from Amgen, Celgene, and Janssen and grants from AstraZeneca, Italian Association for Cancer Research (AIRC-MFA18850), and European Hematology Association outside the submitted work. Y. Kawano reports personal fees from Janssen Pharmaceuticals, Sanofi, Takeda Pharmaceutical Company Limited, Ono Pharmaceutical CO., LTD., and Bristol-Myers Squibb outside the submitted work. I.M. Ghobrial reports personal fees from Bristol-Myers Squibb, Genentech, AbbVie, Janssen, and GlaxoSmithKline outside the submitted work. K.C. Anderson reports personal fees from Pfizer, AstraZeneca, Janssen, Precision Biosciences, Mana, Windmill, Staron, and Raqia, and other support from C4Therapeutics and Oncoprep outside the submitted work. No disclosures were reported by the other authors.

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Authors’ Contributions

G. Bianchi: Conceptualization, resources, formal analysis, funding acquisition, investigation, methodology, writing—original draft.


T. Sebastianian: Resources, investigation. R.D. Carrasquillo: Resources.

I.M. Ghobrial: Resources. K.C. Anderson: Conceptualization, resources, writing—review and editing.

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REFERENCES


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