Clinical and Experimental Medicine (2019) 19:133–141
https://doi.org/10.1007/s10238-018-0531-4

ORIGINAL ARTICLE

RANKL-induced c-Src activation contributes to conventional anti-cancer drug resistance and dasatinib overcomes this resistance in RANK-expressing multiple myeloma cells

Keiji Mashimo[1,2] · Masanobu Tsubaki[1] · Tomoya Takeda[1] · Ryota Asano[1] · Minami Jinushi[1] · Motohiro Imano[3] · Takao Satou[4] · Katsuhiko Sakaguchi[2] · Shozo Nishida[1]

Received: 28 June 2018 / Accepted: 27 September 2018 / Published online: 5 October 2018

Abstract

The survival and growth of multiple myeloma (MM) cells are facilitated by cell–cell interactions with bone marrow stromal cells and the bone marrow microenvironment. These interactions induce de novo drug resistance known as cell adhesion-mediated drug resistance. Our previous results recently revealed that the receptor activator of NF-κB (RANK) ligand (RANKL), which is expressed by bone marrow stromal cells, contributes to anti-cancer drug resistance through the activation of various signaling molecules and suppression of Bim expression in RANK-expressing MM cells. However, the detailed mechanisms underlying RANKL-induced drug resistance remain uncharacterized. In the present study, we investigated the mechanism of RANKL-induced drug resistance in RANK-expressing MM cell lines. We found treatment of MM cells with RANKL-induced c-Src phosphorylation and activation of the downstream signaling molecules Akt, mTOR, STAT3, JNK, and NF-κB. In addition, treatment with dasatinib, a c-Src inhibitor, overcame RANKL- and bone marrow stromal cell-induced drug resistance to adriamycin, vincristine, dexamethasone, and melphalan by suppressing c-Src, Akt, mTOR, STAT3, JNK, and NF-κB activation and enhancing expression of Bim. Overall, RANKL- and bone marrow stromal cell-induced drug resistance correlated with the activation of c-Src signaling pathways, which caused a decrease in Bim expression. Dasatinib treatment of RANK-expressing MM cells re-sensitized them to anti-cancer drugs. Therefore, inhibition of c-Src may be a new therapeutic approach for overcoming RANKL-induced drug resistance in patients with MM.

Keywords Multiple myeloma · RANK · RANKL · Src · Drug resistance

Introduction

Multiple myeloma (MM) is a hematopoietic tumor of clonal plasma cells that proliferate, express a monoclonal immunoglobulin, and accumulate in bone marrow. New chemotherapeutic drugs, including the proteasome inhibitors bortezomib and ixazomib, the immunomodulatory drugs thalidomide and lenalidomide, and the histone deacetylase inhibitor panobinostat, have been developed as treatments for MM and can be used in combination with conventional anti-cancer drugs, such as adriamycin, vincristine, dexamethasone, and melphalan. However, MM is considered incurable, as more than 90% of patients relapse, or do not respond to presently available chemotherapies [1–4]. Therefore, it is important that new approaches overcoming drug resistance be developed for patients with MM.

MM cell survival and growth is facilitated by cell–cell interactions with bone marrow stromal cells and osteoblasts,

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10238-018-0531-4) contains supplementary material, which is available to authorized users.

✉ Shozo Nishida
nishida@phar.kindai.ac.jp

1 Division of Pharmacotherapy, Faculty of Pharmacy, Kindai University, Kowakae, Higashi-Osaka 577-8502, Japan

2 Department of Pharmacy, Japanese Red Cross Society Wakayama Medical Center, Wakayama, Japan

3 Department of Surgery, Faculty of Medicine, Kindai University, Osakasayama, Osaka, Japan

4 Department of Pathology, Faculty of Medicine, Kindai University, Osakasayama, Osaka, Japan

and cytokine autocrine loops in the bone marrow microenvironment [5–11]. Bone marrow stromal cells and osteoblasts express extracellular matrix components, such as fibronectin, vimentin, and collagen. MM cells adhere to this extracellular matrix via integrin β1 [12]. Interactions with bone marrow stromal cells and osteoblasts via adhesion molecules induce various signaling pathways that regulate the expression of drug resistance-related factors, including ATP-binding cassette transporters, and Bcl-2 and IAP family proteins [12–14]. These interactions induce de novo drug resistance known as cell adhesion-mediated drug resistance. In addition, innate and/or de novo drug resistance influences the bone marrow microenvironment, where long-term exposure to anti-cancer drugs results in acquired resistance by MM cells [15]. MM cells from patients and human MM cell lines express receptor activator of NF-κB (RANK), and patients with International Staging System stages II and III MM tend to overexpress RANK by approximately 15%, where normal expression of RANK occurs in more than 95% of MM patients [16, 17]. RANK ligand (RANKL) is expressed by bone marrow stromal cells and osteoblasts [11, 18–21]. RANK binds to RANKL and induces activation of signaling pathways, such as the mitogen-activated protein kinase (MAPK), (MEK)/extracellular signal-regulated kinase 1/2 (ERK1/2), c-Jun N-terminal kinase (JNK), p38MAPK, nuclear factor-kappa B (NF-κB), and phosphatidylinositol 3-kinase (PI3 K)/Akt/mammalian target of rapamycin (mTOR) pathways [22]. Our previous study found that RANKL induces drug resistance in RANK-expressing MM cells via activation of Akt, mTOR, JNK, NF-κB, and STAT3. Quadruple- or quintuple-signaling inhibitor combinations were needed to overcome this drug resistance [23]. However, the detailed mechanisms of RANKL-induced drug resistance remain unclear. In the present study, we investigated the mechanisms of RANKL-induced drug resistance in RANK-expressing MM cell lines. RANKL induced c-Src phosphorylation and activation of downstream signaling pathways, such as Akt, mTOR, STAT3, JNK, and NF-κB. Furthermore, inhibition of the c-Src signaling pathway using dasatinib overcame the RANKL- and bone marrow stromal cell-induced drug resistance. Therefore, our data suggest that c-Src plays an important role in RANKL-induced drug resistance, and Src inhibitors such as dasatinib can overcome this resistance.

Materials and methods

Materials

Melphalan was purchased from Sigma (St. Louis, MO, USA). Dexamethasone was purchased from Wako (Tokyo, Japan). Dasatinib was purchased from Cayman Chemical (Ann Arbor, MI, USA). These reagents were dissolved in dimethyl sulfoxide, diluted in phosphate-buffered saline (0.05 M, pH 7.4), filtered through 0.45-μm syringe filters (IWAKI GLASS, Tokyo, Japan), and then used in assays as described below.

Adriamycin and vincristine were purchased from Sigma. Human soluble RANKL was purchased from PeproTech (London, UK). These reagents were dissolved in phosphate-buffered saline (0.05 M, pH 7.4) and used in assays as described below.

Cell culture

The RANK-expressing MM cell lines ARH-77, IM9, and RPMI8226 were used in this study. ST2 cells were obtained from Riken Cell Bank (Ibaraki, Japan). All cells were cultured in RPMI1640 medium (Sigma) supplemented with 10% fetal calf serum (Gibco, Carlsbad, CA, USA), 100 μg/ml penicillin (Gibco), 100 U/ml streptomycin (Gibco), and 25 mM HEPES (pH 7.4; Wako), and were maintained in an atmosphere containing 5% CO_2.

Trypan blue dye exclusion assay

The effect of various anti-cancer drugs on cell survival/proliferation was determined using the trypan blue dye exclusion assay as described previously [23].

Western blotting

Proteins in cellular, cytoplasmic, and nuclear fractions were extracted using the ProteoExtract Subcellular Proteome Extraction Kit (Calbiochem, San Diego, CA, USA). Lysate protein content was quantified using a BCA protein assay kit. Extracts (20 μg of protein) were fractionated on sodium dodecyl sulfate–polyacrylamide gels and transferred to polyvinylidene fluoride membranes (GE Healthcare, Buckinghamshire, UK). The membranes were blocked with a 3% skim milk solution and incubated overnight at 4 °C with the following antibodies: anti-NF-κB p65 (#3034), anti-phospho-Akt (#9271), anti-phospho-mTOR (#2971), anti-phospho-STAT3 (#9131), anti-phospho-JNK (#9251), anti-Akt (#9271), anti-mTOR (#2972), anti-STAT3 (#9132), and anti-JNK (#9252), which were purchased from Cell Signaling Technology (Beverly, MA, USA), as well as anti-phospho-c-Src (9A6), anti-c-Src (N-16), and anti-Bim (H191) purchased from Santa Cruz Biotechnology (CA, USA). Subsequently, the membranes were incubated with horseradish peroxidase-coupled sheep anti-rabbit IgG (Amersham) for 1 h at room temperature. The reactive proteins were visualized using Luminata Forte (Merck Millipore, Billerica, MA, USA) according to the manufacturer's instructions. As an internal standard, β-actin and lamin proteins were detected

using anti-β-actin mouse monoclonal primary antibody (Sigma) and anti-lamin rabbit polyclonal primary antibody (Santa Cruz Biotechnology).

Statistical analysis

All results are expressed as mean ± standard deviation for several independent experiments. Multiple comparisons were performed for data using ANOVA with Dunnett's post-test. A p value of less than 5% was considered statistically significant.

Results

RANKL-induced c-Src phosphorylation in MM cells

RANK activation by RANKL stimulation promotes activation of signaling molecules, such as Akt, mTOR, JNK, and NF-κB, through TRAF-2 and TRAF-6 mediation that results in c-Src activation in osteoclasts and other cells [22, 24]. We investigated whether phosphorylation of c-Src by RANKL is important for downstream RANK signaling. RANKL was found to induce c-Src activation in RANK-expressing IM9, ARH-77, and RPM8226 cells (Fig. 1).

Src inhibitor suppressed the RANKL-induced signal molecules activation in MM cells

Our previous study showed that RANKL stimulation promotes the phosphorylation of Akt, mTOR, STAT3, and JNK, and enhances the nuclear localization of NF-κB in MM cells [23]. Thus, we examined whether the activation of c-Src through RANKL treatment would promote the activation of Akt, mTOR, JNK, NF-κB, and STAT3. We found that dasatinib, a c-Src inhibitor, suppressed Akt, mTOR, JNK, and STAT3 phosphorylation, as well as nuclear translocation of NF-κB (Fig. 2). Our previous study suggested that RANK/RANKL-mediated drug resistance is involved in the downregulation of Bim [23]. Inhibition of c-Src activation by dasatinib dampened RANKL-induced Bim downregulation in IM9, ARH-77, and RPMI8226 cells (Fig. 2).

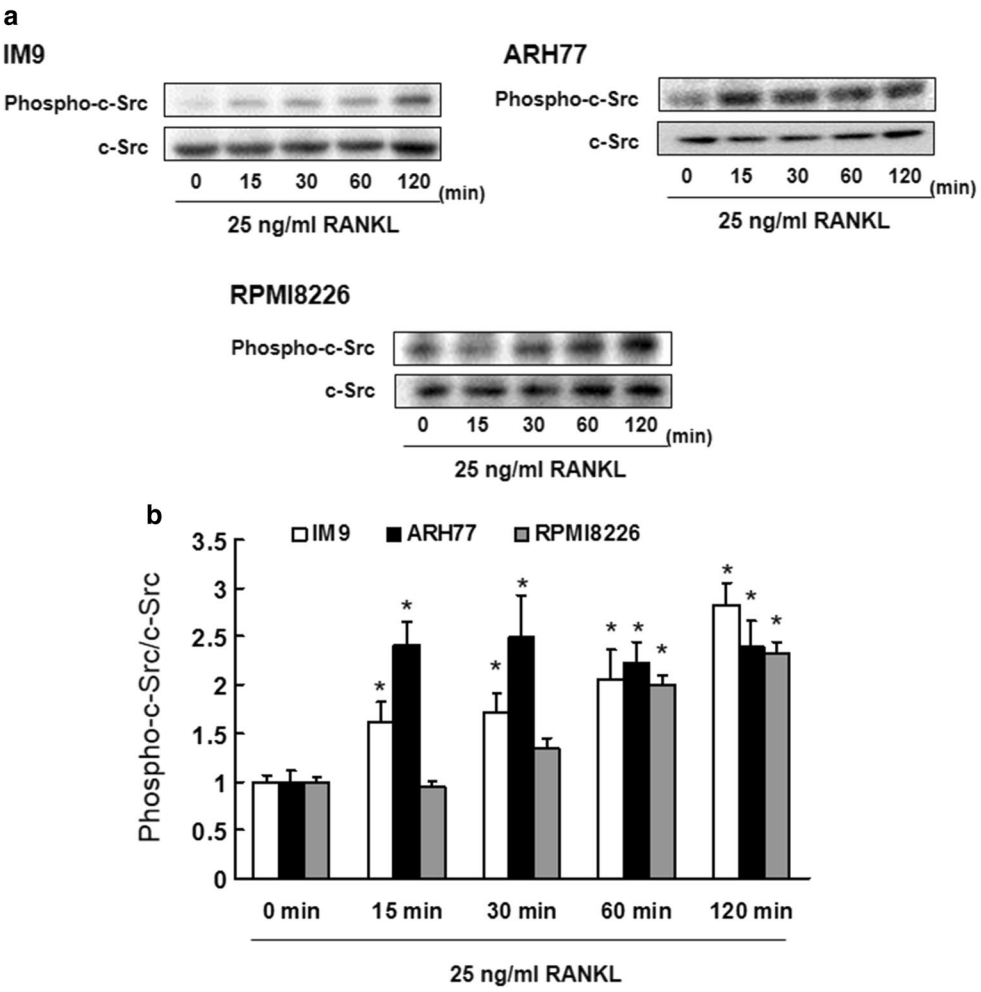

Fig. 1 RANKL activates c-Src in RANK-positive MM cells. **a** Whole-cell lysates were prepared and immunoblotted with antibodies against phosphorylated c-Src (phospho-c-Src) and c-Src in IM9, ARH77, or RPMI8226 cells. **b** Quantification of the amounts of phospho-c-Src, after normalization to the amounts of total c-Src protein. *$p < 0.01$ versus control (0 min) (ANOVA with Dunnett's test)

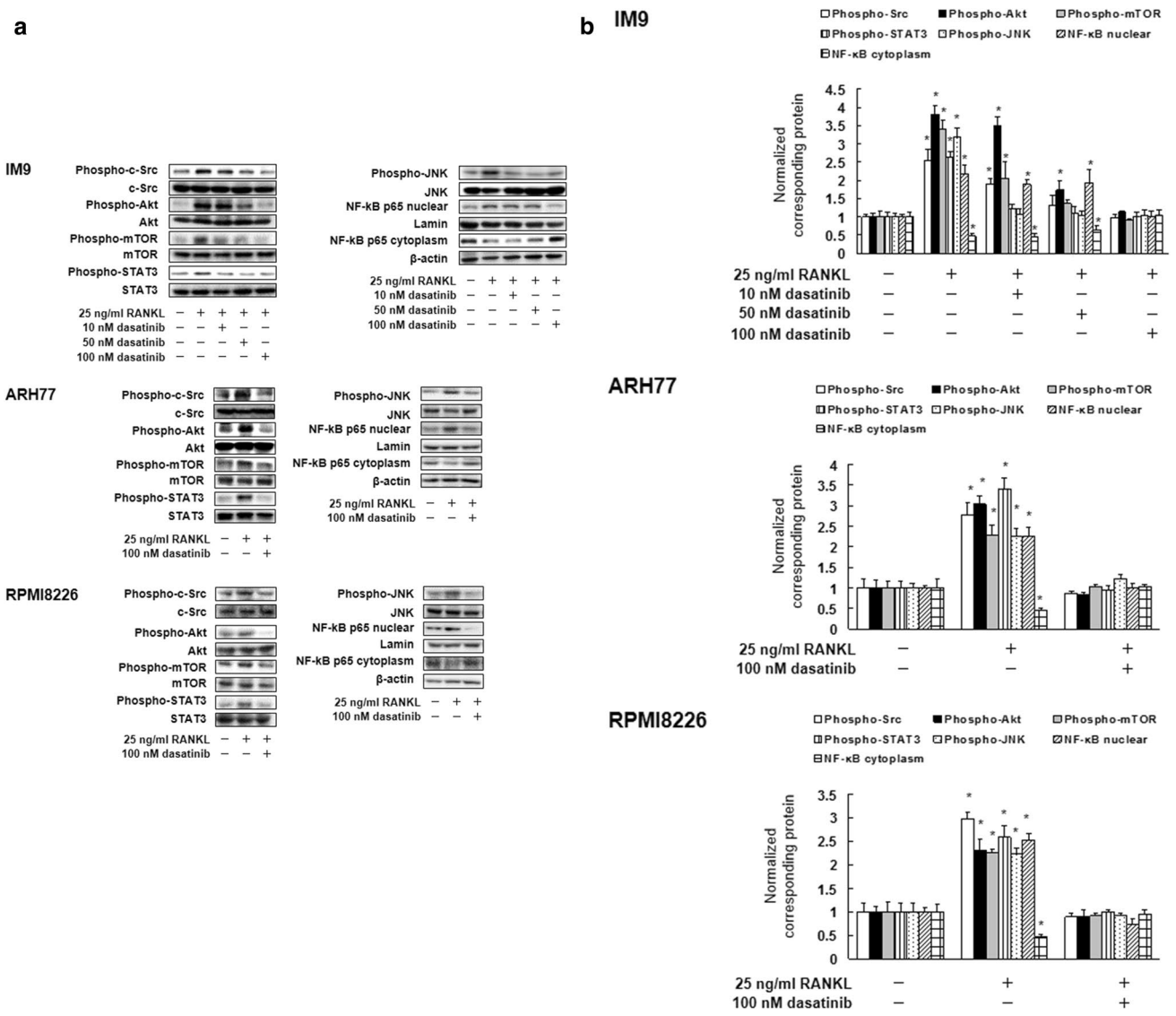

Fig. 2 Effect of dasatinib on RANKL-regulated c-Src, Akt, mTOR, STAT3, JNK and NF-κB activation, and Bim expression in RANK-expressing MM cells. **a** IM9, ARH77, and RPMI8226 cells were exposed to the indicated concentrations of dasatinib. After incubation with dasatinib for 24 h, cells were treated with RANKL for 60 min. Whole-cell lysates were prepared and immunoblotted with antibodies against phosphorylated c-Src (phospho-c-Src), phosphorylated Akt (phospho-Akt), phosphorylated mTOR (phospho-mTOR), phosphorylated JNK (phospho-JNK), phosphorylated STAT3 (phospho-STAT3), NF-κB, Akt, mTOR, JNK, STAT3, β-actin, and Lamin. **b** Quantification of the amounts of phospho-c-Src, phospho-Akt, phospho-mTOR, phospho-JNK, phospho-STAT3, or NF-κB, after normalization to the amounts of corresponding protein. $*p < 0.01$ versus control (ANOVA with Dunnett's test). **c** IM9, ARH77, and RPMI8226 cells were exposed to the indicated concentrations of dasatinib. After incubation with dasatinib for 24 h, cells were treated with RANKL for 48 h. Whole-cell lysates were prepared and immunoblotted with antibodies against Bim and β-actin. **d** Quantification of the amounts of Bim, after normalization to the amounts of β-actin protein. $*p < 0.01$ versus control (0 min) (ANOVA with Dunnett's test)

Dasatinib overcame RANKL- and bone marrow stromal cell-induced drug resistance

Dasatinib was found to suppress RANKL-induced activation of the c-Src signaling pathway and Bim downregulation. Therefore, we treated IM9, ARH-77, and RPMI8226 cells with dasatinib in the presence or absence of RANKL stimulation in order to determine whether sensitivity to the conventional anti-cancer drugs adriamycin, vincristine, dexamethasone, and melphalan could be restored in cells with RANKL-induced drug resistance. Treatment with dasatinib at concentrations that inhibit the Src signaling pathway (10-100 nM) did not affect the viability of IM9, ARH-77, and RPMI8226 cells (Suppl. Fig. 1). Combination of dasatinib with adriamycin, vincristine, dexamethasone, or melphalan significantly induced cell death similar

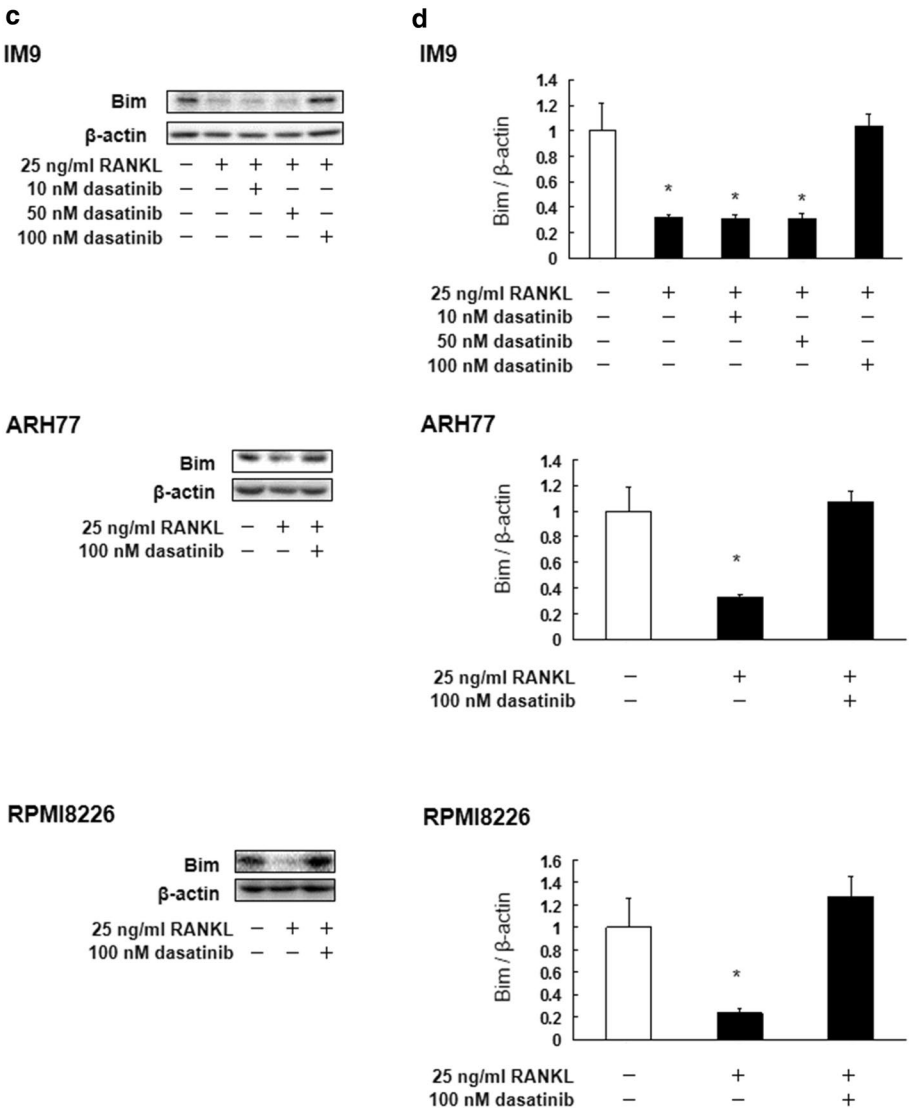

Fig. 2 (continued)

to conventional drugs alone (no treatment with RANKL) (Fig. 3). Thus, dasatinib re-sensitized RANKL-induced drug-resistant IM9, ARH-77, and RPMI8226 cells to adriamycin, vincristine, dexamethasone, and melphalan.

Next, we investigated whether dasatinib can reverse bone marrow stromal cell-induced drug resistance. Co-culturing of IM9, ARH-77, or RPMI8226 cells with bone marrow stromal cells made them resistant to adriamycin, vincristine, dexamethasone, and melphalan. Dasatinib re-sensitized bone marrow stromal cell-induced drug-resistant IM9, ARH-77, and RPMI8226 cells to adriamycin, vincristine, dexamethasone, and melphalan (Fig. 4).

These data suggest a critical role for c-Src activation in RANKL-mediated drug resistance in RANK-expressing MM cells.

Discussion

In the present study, we demonstrated RANKL-induced drug resistance in the RANK-expressing MM cell lines IM9, ARH-77, and RPMI8226 by activating c-Src and its downstream signaling molecules Akt, mTOR, STAT3, JNK, and NF-κB, which triggers decreased expression of Bim. In addition, treatment with dasatinib at concentrations that did not affect IM9, ARH-77, and RPMI8226 cell viability overcame RANKL-mediated drug resistance in RANK-expressing MM cells. RANKL binds to its cognate receptor, RANK, following the activation of several signaling pathways, including the PI3 K/Akt, JAK/STAT, MAPK (ERK1/2 and JNK), and NF-κB pathways [22].

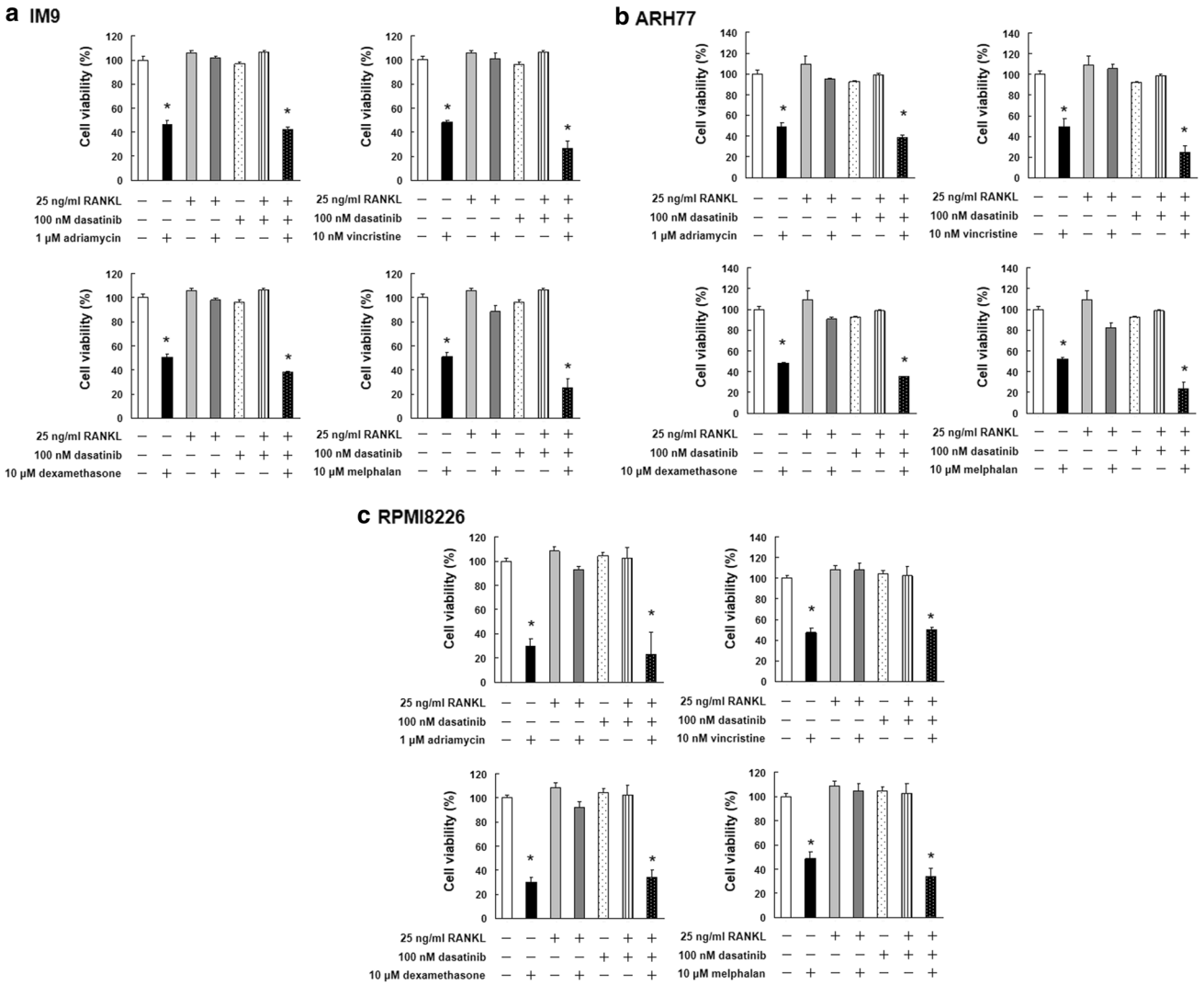

Fig. 3 Effect of dasatinib on RANKL-mediated drug resistance. **a** IM9, **b** ARH-77, and **c** RPMI8226 cells were treated with 100 nM dasatinib. After 12 h, cells were treated with RANKL for 24 h. Cells were subsequently exposed to the indicated concentrations of doxorubicin, vincristine, dexamethasone, or melphalan. After incubation for 72 h, the number of dead cells was measured by trypan blue staining. The results are representative of five independent experiments. *$p < 0.01$ versus untreated IM9, ARH-77, or RPMI8226 cells (analysis of variance with Dunnett's test)

RANK also binds to TRAF-2 and TRAF-6 via its cytoplasmic domain. Subsequently, TRAF-2 and TRAF-6 promote c-Src phosphorylation [22, 24], which in turn results in the activation of the PI3 K/Akt, MAPK, STAT3, and NF-κB pathways [25]. The findings of the present study suggest that RANKL binds to RANK, thereby facilitating the activation of c-Src and its downstream signaling molecules, including Akt, mTOR, STAT3, JNK, and NF-κB. It has been reported that the activation of the c-Src/STAT3 and c-Src/Akt pathways through β1 integrin adhesion to fibronectin is involved in cell adhesion-mediated drug resistance in MM cells [26]. In addition, interactions between macrophages and MM cells via P-selectin glycoprotein ligand-1/selectin and ICAM-1/CD18 activate the c-Src pathway and suppress caspase activation by melphalan, adriamycin, and bortezomib in MM cells [27]. It has also been indicated that acquired multidrug resistance in MM cells acts, at least in part, through the activation of c-Src, where treatment with c-Src inhibitors re-sensitizes cells to various anti-cancer drugs [14]. Moreover, it has been suggested that c-Src activation is involved in the resistance to conventional anti-cancer drugs and molecular targeting drugs used to treat various cancers, such as lung, breast, and gastric cancers, and chronic myeloid leukemia [28–31]. Our previous study indicated that RANKL-mediated drug resistance is associated with the activation of Akt, mTOR, STAT3, JNK, and NF-κB in RANK-expressing MM cells, and suppression of Bim expression [23].

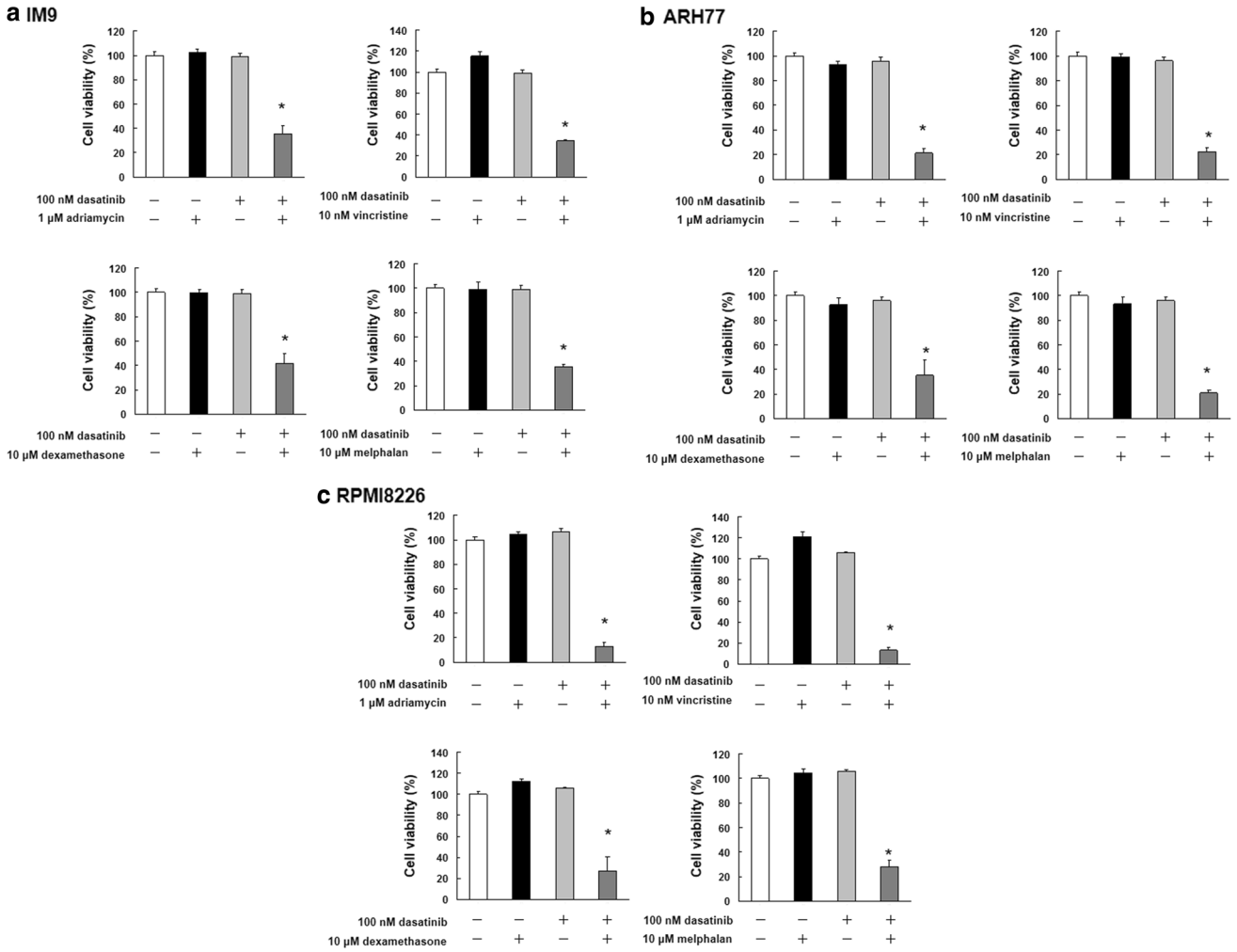

Fig. 4 Effect of dasatinib on bone marrow stromal cells-mediated drug resistance. **a** IM9, **b** ARH-77, and **c** RPMI8226 cells were treated with 100 nM dasatinib. After 12 h, cells were co-cultured with bone marrow stromal cells for 24 h. Cells were subsequently exposed to the indicated concentrations of doxorubicin, vincristine, dexamethasone, or melphalan. After incubation for 72 h, the number of dead cells was measured by trypan blue staining. The results are representative of five independent experiments. *$p < 0.01$ versus controls (analysis of variance with Dunnett's test)

SAHA, a histone deacetylase inhibitor, re-sensitizes cells to adriamycin, vincristine, dexamethasone, and melphalan by enhancing Bim expression [23]. These results suggest that the activation of the Src signaling pathway contributes to RANKL- and bone marrow stromal cell-induced drug resistance in RANK-expressing MM cells.

Dasatinib is a multi-kinase inhibitor that competes for ATP with Src/Abl, c-Kit, EphA2, and platelet-derived growth factor receptor β [32, 33]. It has been shown that constitutive activation of Src promotes proliferation and survival in MM cells, and inhibition of Src by dasatinib suppresses tumor growth in mouse models [32, 34, 35], but dasatinib alone has minimal effect in MM patients [36]. It has also been reported that dasatinib enhances the induction of apoptosis of conventional anti-myeloma agents in MM cell lines and plasma cells from MM patients [33]. Therefore, dasatinib is administered to human patients in doses of 70–140 mg, and administration of dasatinib (70 mg) has a C_{max} serum concentration of approximately 100–200 nM [37]. In addition, it has been reported that dasatinib plasma concentrations reach approximately 200–400 nM without displaying dose-limiting toxicity in pediatric patients [37, 38]. These findings suggest that dasatinib may prove useful in combination pharmacological therapies for treating RANK-mediated drug-resistant MM.

In conclusion, this study indicates that dasatinib can overcome RANKL- and bone marrow stromal cell-induced drug resistance by suppressing the Src signaling pathway and enhancing Bim expression in RANK-expressing MM cells. These findings may prove to be useful in the development of inhibitors of RANK-mediated drug resistance, such as dasatinib, for targeting of MM cells.

Acknowledgements This work was supported in part by a Grant-in-Aid for Scientific Research (C) (Grant Number 15K08116), Grant-in-Aid for Young Scientists (B) (Grant Number 16K18965) from the Japan Society for the Promotion of Science (JSPS) and by Ministry of Education, Culture, Sports, Science, and Technology (MEXT)-Supported Program for the Strategic Research Foundation at Private Universities, 2014-2018 (Grant number S1411037).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Ludwig H, Milosavljevic D, Zojer N, et al. Immunoglobulin heavy/light chain ratios improve paraprotein detection and monitoring, identify residual disease and correlate with survival in multiple myeloma patients. Leukemia. 2013;27(1):213–9.
2. Tsubaki M, Satou T, Itoh T, et al. Overexpression of MDR1 and survivin, and decreased Bim expression mediate multidrug-resistance in multiple myeloma cells. Leuk Res. 2012;36(10):1315–22.
3. Kumar SK, Rajkumar SV, Dispenzieri A, et al. Improved survival in multiple myeloma and the impact of novel therapies. Blood. 2008;111(5):2516–20.
4. Mitsiades CS, Hayden PJ, Anderson KC, et al. From the bench to the bedside: emerging new treatments in multiple myeloma. Best Pract Res Clin Haematol. 2007;20(4):797–816.
5. Meads MB, Hazlehurst LA, Dalton WS. The bone marrow microenvironment as a tumor sanctuary and contributor to drug resistance. Clin Cancer Res. 2008;14(9):2519–26.
6. Tsubaki M, Takeda T, Sakamoto K, et al. Bisphosphonates and statins inhibit expression and secretion of MIP-1α via suppression of Ras/MEK/ERK/AML-1A and Ras/PI3K/Akt/AML-1A pathways. Am J Cancer Res. 2014;5(1):168–79.
7. Tsubaki M, Takeda T, Tomonari Y, et al. The MIP-1α autocrine loop contributes to decreased sensitivity to anticancer drugs. J Cell Physiol. 2018;233(5):4258–71.
8. Tsubaki M, Mashimo K, Takeda T, et al. Statins inhibited the MIP-1α expression via inhibition of Ras/ERK and Ras/Akt pathways in myeloma cells. Biomed Pharmacother. 2016;78:23–9.
9. Tsubaki M, Komai M, Itoh T, et al. Inhibition of the tumour necrosis factor-alpha autocrine loop enhances the sensitivity of multiple myeloma cells to anticancer drugs. Eur J Cancer. 2013;49(17):3708–17.
10. Tsubaki M, Kato C, Nishinobo M, et al. Nitrogen-containing bisphosphonate, YM529/ONO-5920, inhibits macrophage inflammatory protein 1 alpha expression and secretion in mouse myeloma cells. Cancer Sci. 2008;99(1):152–8.
11. Tsubaki M, Kato C, Manno M, et al. Macrophage inflammatory protein-1alpha (MIP-1alpha) enhances a receptor activator of nuclear factor kappaB ligand (RANKL) expression in mouse bone marrow stromal cells and osteoblasts through MAPK and PI3 K/Akt pathways. Mol Cell Biochem. 2007;304(1–2):53–60.
12. Abdi J, Chen G, Chang H. Drug resistance in multiple myeloma: latest findings and new concepts on molecular mechanisms. Oncotarget. 2013;4(12):2186–207.
13. Tsubaki M, Takeda T, Ogawa N, et al. Overexpression of survivin via activation of ERK1/2, Akt, and NF-κB plays a central role in vincristine resistance in multiple myeloma cells. Leuk Res. 2015;39(4):445–52.
14. Tsubaki M, Komai M, Itoh T, et al. By inhibiting Src, verapamil and dasatinib overcome multidrug resistance via increased expression of Bim and decreased expressions of MDR1 and survivin in human multidrug-resistant myeloma cells. Leuk Res. 2014;38(1):121–30.
15. Furukawa Y, Kikuchi J. Epigenetic mechanisms of cell adhesion-mediated drug resistance in multiple myeloma. Int J Hematol. 2016;104(3):281–92.
16. Sung B, Cho SG, Liu M, et al. Butein, a tetrahydroxychalcone, suppresses cancer-induced osteoclastogenesis through inhibition of receptor activator of nuclear factor-kappaB ligand signaling. Int J Cancer. 2011;129(9):2062–72.
17. Sampaio MS, Vettore AL, Yamamoto M, et al. Expression of eight genes of nuclear factor-kappa B pathway in multiple myeloma using bone marrow aspirates obtained at diagnosis. Histol Histopathol. 2009;24(8):991–7.
18. Nishida S, Tsubaki M, Hoshino M, et al. Nitrogen-containing bisphosphonate, YM529/ONO-5920 (a novel minodronic acid), inhibits RANKL expression in a cultured bone marrow stromal cell line ST2. Biochem Biophys Res Commun. 2005;328(1):91–7.
19. Tsubaki M, Kato C, Isono A, et al. Macrophage inflammatory protein-1α induces osteoclast formation by activation of the MEK/ERK/c-Fos pathway and inhibition of the p38MAPK/IRF-3/IFN-β pathway. J Cell Biochem. 2010;111(6):1661–72.
20. Tsubaki M, Komai M, Itoh T, et al. Nitrogen-containing bisphosphonates inhibit RANKL- and M-CSF-induced osteoclast formation through the inhibition of ERK1/2 and Akt activation. J Biomed Sci. 2014;21:10.
21. Tsubaki M, Satou T, Itoh T, et al. Bisphosphonate- and statin-induced enhancement of OPG expression and inhibition of CD9, M-CSF, and RANKL expressions via inhibition of the Ras/MEK/ERK pathway and activation of p38MAPK in mouse bone marrow stromal cell line ST2. Mol Cell Endocrinol. 2012;361(1–2):219–31.
22. Liu H, Tamashiro S, Baritaki S, et al. TRAF6 activation in multiple myeloma: a potential therapeutic target. Clin Lymphoma Myeloma Leuk. 2012;12(3):155–63.
23. Tsubaki M, Takeda T, Yoshizumi M, et al. RANK-RANKL interactions are involved in cell adhesion-mediated drug resistance in multiple myeloma cell lines. Tumour Biol. 2016;37(7):9099–110.
24. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature. 2003;423(6937):337–42.
25. Frame MC. Src in cancer: deregulation and consequences for cell behaviour. Biochim Biophys Acta. 2002;1602(2):114–30.
26. Lin L, Yan F, Zhao D, et al. Reelin promotes the adhesion and drug resistance of multiple myeloma cells via integrin β1 signaling and STAT3. Oncotarget. 2016;7(9):9844–58.
27. Zheng Y, Yang J, Qian J, et al. PSGL-1/selectin and ICAM-1/CD18 interactions are involved in macrophage-induced drug resistance in myeloma. Leukemia. 2013;27(3):702–10.
28. Kanda R, Kawahara A, Watari K, et al. Erlotinib resistance in lung cancer cells mediated by integrin β1/Src/Akt-driven bypass signaling. Cancer Res. 2013;73(20):6243–53.
29. Wu ZH, Lin C, Liu MM, et al. Src inhibition can synergize with gemcitabine and reverse resistance in triple negative Breast cancer cells via the AKT/c-Jun pathway. PLoS ONE. 2016;11(12):e0169230.
30. Nam HJ, Im SA, Oh DY, et al. Antitumor activity of saracatinib (AZD0530), a c-Src/Abl kinase inhibitor, alone or in combination with chemotherapeutic agents in gastric cancer. Mol Cancer Ther. 2013;12(1):16–26.
31. Ferreira PA, Ruela-de-Sousa RR, Queiroz KC, et al. Knocking down low molecular weight protein tyrosine phosphatase (LMW-PTP) reverts chemoresistance through inactivation of Src and Bcr-Abl proteins. PLoS ONE. 2012;7(9):e44312.
32. Coluccia AM, Cirulli T, Neri P, et al. Validation of PDGFRbeta and c-Src tyrosine kinases as tumor/vessel targets in patients with

multiple myeloma: preclinical efficacy of the novel, orally available inhibitor dasatinib. Blood. 2008;112(4):1346–56.
33. de Queiroz Crusoe E, Maiso P, Fernandez-Lazaro D, et al. Transcriptomic rationale for the synergy observed with dasatinib + bortezomib + dexamethasone in multiple myeloma. Ann Hematol. 2012;91(2):257–69.
34. Ishikawa H, Tsuyama N, Abroun S, et al. Requirements of src family kinase activity associated with CD45 for myeloma cell proliferation by interleukin-6. Blood. 2002;99(6):2172–8.
35. Ishikawa H, Tsuyama N, Abroun S, et al. Interleukin-6, CD45 and the src-kinases in myeloma cell proliferation. Leuk Lymphoma. 2003;44(9):1477–81.
36. Wildes TM, Procknow E, Gao F, et al. Dasatinib in relapsed or plateau-phase multiple myeloma. Leuk Lymphoma. 2009;50(1):137–40.
37. Futosi K, Németh T, Pick R, et al. Dasatinib inhibits proinflammatory functions of mature human neutrophils. Blood. 2012;119(21):4981–91.
38. Aplenc R, Blaney SM, Strauss LC, et al. Pediatric phase I trial and pharmacokinetic study of dasatinib: a report from the children's oncology group phase I consortium. J Clin Oncol. 2011;29(7):839–44.