Biochemical and Biophysical Research Communications 391 (2010) 310–315

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Cytoprotective effect of imatinib mesylate in non-BCR-ABL-expressing cells along with autophagosome formation

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ARTICLE INFO

Article history:
Received 6 November 2009
Available online 11 November 2009

Keywords:
Imatinib mesylate
Tyrosine kinase inhibitor
Chronic myelogenous leukemia
Autophagy
ER stress

ABSTRACT

Treatment with imatinib mesylate (IM) results in an increased viable cell number of non-BCR-ABL-expressing cell lines by inhibiting spontaneous apoptosis. Electron microscopy revealed an increase of autophagosomes in response to IM. IM attenuated the cytotoxic effect of cytosine arabinoside, as well as inhibiting cell death with serum-deprived culture. Cytoprotection with autophagosome formation by IM was observed in various leukemia and cancer cell lines as well as normal murine embryonic fibroblasts (MEFs). Complete inhibition of autophagy by knockdown of *atg5* in the Tet-off *atg5*$^{-/-}$ MEF system attenuated the cytoprotective effect of IM, indicating that the effect is partially dependent on autophagy. However, cytoprotection by IM was not mediated through suppression of ROS production via mitophagy, ER stress via ribophagy, or proapoptotic function of ABL kinase. Although the target tyrosine kinase(s) of IM remains unclear, our data provide novel therapeutic possibilities of using IM for cytoprotection.

Introduction

Chronic myelogenous leukemia (CML) is caused by translocation of chromosomes 9 and 22 in hematopoietic progenitor cells. This genetic event results in production of an abnormal fusion BCR-ABL protein in which constitutive activation of tyrosine kinase of ABL mimics the cell growth or survival signals of various growth factors, inducing a growth advantage of the CML clone in bone marrow. Imatinib mesylate (IM) was developed as a specific inhibitor of BCR-ABL kinase activity by binding to the ATP-binding pocket of ABL kinase [1]. Since IM was also found to inhibit other tyrosine kinases (e.g., ABL, KIT, and PDGF receptor), it has been clinically used for treating gastrointestinal tumors (GISTs) with the ligand-independent constitutive activation of KIT by mutations of exon 11 and treatment of chronic eosinophilic leukemia with constitutive activation of the FIP1L1/PDGFRα fusion protein by interstitial deletion of chromosome 4q12 [2,3]. Dramatic therapeutic response to IM in CML patients has become the first model of "target therapy" for cancer patients, using a low molecular weight chemical compound. IM therapy has now become the first-line treatment for patients with CML [4].

Autophagy is an intracellular degradation system that delivers cytoplasmic constitutions to the lysosome. Intracellular proteins and organelle are sequestered by the autophagosome, then delivered to the lysosome and degraded [5]. Autophagy is a process by which cells can adapt their metabolism to starvation nutrients or is a typical consequence of loss of growth factor signals, allowing cells to evade programmed cell death. However, in addition to its role in the starvation process, accumulating evidence suggests that autophagy is closely related to intracellular quality control of proteins and organelle, neurodegeneration, immune response, tumor suppression, anti-aging, and cell death [6].

Recently, it has been reported that IM induces autophagy in various cell lines [7]. Combining IM with an autophagy inhibitor such as chloroquine has been reported to enhance elimination of CML clones *in vitro* and *in vivo* [8]. This result suggests that targeting autophagy may provide therapeutic benefit in IM therapy for CML. Interestingly, a recent report demonstrated that IM has a therapeutic effect on diabetes mellitus, although the underlying mechanism is still unclear [9].

We here report that IM has a potent cytoprotective effect, along with autophagosome formation, in non-BCR-ABL-expressing cell lines and normal murine embryonic fibroblasts (MEFs).

Materials and methods

Cell lines and reagents. Cell lines such as K562 (a CML cell line expressing p210BCR-ABL), HL-60 and U937 (acute myelogenous

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doi:10.1016/j.bbrc.2009.11.055

leukemia (AML) cell lines), A549 and H226 (lung cancer cell lines), and COLO202 and DLD1 (colon cancer cell lines) were all obtained from the American Type Culture Collection (Rockville, MD) and were maintained in continuous culture in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 10% FBS (Hyclone, Logan, UT), 2 mM L-glutamine, penicillin (50 U/ml), and streptomycin (100 μg/ml). The IM was provided by Novartis Pharma AG (Basel, Switzerland) and cytosine arabinoside (Ara-C) was provided by Nippon Shinyaku Co., Ltd. (Kyoto, Japan). E-64-d and pepstatin A, which are inhibitors for lysosomal proteases, were purchased from BIOMOL International L.P. (Plymouth Meeting, PA). Tunicamycin (TN) and trehalose were purchased from Sigma–Aldrich (St. Louis, MO), and rapamycin was obtained from Calbiochem (Darmstadt, Germany).

Assessment of viable cell number and morphology of cultured cells. The viable cell number was assessed by CellTiter Blue, a cell viability assay kit (Promega Co., Madison, WI), with fluorescence measurements at 570 nm for excitation and 590 nm for fluorescence emission. For morphologic assessment, the cell suspensions were sedimented and fixed on slide glasses using a Shandon Cytospin II (Shandon, Pittsburgh, PA), and preparations were stained with May–Grünwald–Giemsa.

Immunoblotting. Immunoblotting was performed as previously described [10]. In short, cells were lysed with 1% NP-40 Lysis Buffer. Cellular proteins were quantified using a Protein Assay kit of Bio-Rad (Richmond, CA). Equal amounts of proteins were loaded onto the gels, separated by SDS–PAGE and transferred onto Immobilone-P membrane (Millipore Corporation, Billerica, MA). RIPA Lysis Buffer (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was used to extract nuclear protein such as CHOP (GADD153). The membranes were probed with antibodies (Abs) such as anti-microtubule-associated protein 1 light chain 3B (LC3B) Ab (Novus Biologicals, Inc., Littleton, CO), anti-CHOP monoclonal (m) Ab, anti-Atg5 Ab, anti-p62 (sequestosome-1) mAb (Cell Signaling Technology, Danvers, MA), and anti-ABL mAb and anti-β-actin mAb (Santa Cruz Biotechnology). Immunoreactive proteins were detected with horseradish peroxidase-conjugated second mAbs and an enhanced chemiluminescence reagent (ECL) (Amersham Biosciences Corporation). Densitometry was performed using a Molecular Imager, ChemiDoc XRS system (Bio-Rad).

Electron microscopy. Cells were treated with or without IM and fixed with a solution containing 3% glutaraldehyde plus 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.3) for 1 h. The samples were further post-fixed in 1% OsO_4 in the same buffer for 1 h, and subjected to electron microscopic analysis using electron microscope H-7000 (Hitachi, Tokyo, Japan) as described previously [10].

Flow cytometry. Flow cytometry was performed to assess mitochondrial volume and mitochondrial superoxide production. After staining the cells with MitoTracker (Molecular Probes, Inc., Eugene, OR) and MitoSOX (Molecular Probes, Inc.), fluorescence intensities were assessed using a flow cytometer Partec PASI with a Particle Analyzing System (Partec GmbH, Münster, Germany). For cell-cycle analysis, cellular DNAs were stained using the CyStain DNA step2 kit (Partec GmbH) and processed for flow cytometry.

Tet-off system with an atg5$^{-/-}$ mouse embryonic fibroblasts (MEFs). The m5–7 cell line, the *atg5* Tet-off MEF system, was a kind gift from Dr. Noboru Mizushima (Tokyo Medical and Dental University School of Health Science, Tokyo, Japan). Culture conditions for continuous maintenance and for knockdown of *atg5* gene for inhibiting autophagy was previously described in detail [11].

Transfection of c-abl shRNA. Gene silence of *c-abl* in HL-60 cells was performed using *c-abl* shRNA lentiviral particles (sc-29843-V) containing the resistance gene for puromycin (Santa Cruz Biotechnology, Inc.) according to the manufacturer's instruction manual. Transfectants were selected by culturing RPMI 1640 medium containing 5 μg/ml of puromycin and 10% FBS. Cloning of a transfectant with the lower ABL expression by *c-abl* shRNA was performed by limiting dilution. Expression of ABL protein was assessed by immunoblotting with anti-ABL mAb.

Statistics. All data are given as means ± SD. Statistical analysis was performed by using Mann–Whitney's *U* test (two-tailed). The criterion for statistical significance was taken as $p < 0.05$.

Results and discussion

IM induces a cytoprotective effect in non-BCR-ABL-expressing cells

When p210BCR-ABL-expressing K562 cells were treated with IM, the viable cell number was decreased, along with the typical morphological feature of apoptosis such as fragmented nucleus, as previously described (Fig. 1A) [12]. Surprisingly, treatment of HL-60 cells, an non-BCR-ABL-expressing AML cell line, with IM resulted in an increase in viable cell number, compared with the cells cultured in RPMI 1640 medium containing 10% FBS (Supplementary Fig. S1A). When HL-60 cells were cultured in the presence of 3–30 μM of IM for 24–96 h, up to a 1.6-fold increase in the viable cell number was observed in a dose-dependent manner (Fig. 1B). Cell-cycle analysis revealed no effects on cell-cycle progression after treatment with IM (Supplementary Fig. S1B). A few percent of HL-60 cells underwent spontaneous apoptosis, as indicated by the arrows in Fig. 1A, even when cultured in complete medium containing 10% FBS. Since culture of HL-60 cells with IM significantly reduced these apoptotic cells, the increased cell number appeared to be due to inhibition of spontaneous cell death but not by cell-cycle progression. Cytosine arabinoside (Ara-C), a representative chemotherapeutic drugs for AML, inhibited growth of HL-60 cells in a dose-dependent manner. However, cell-growth inhibition by Ara-C was significantly attenuated in the presence of IM at the lower concentrations of Ara-C (Fig. 1C). Furthermore, when HL-60 cells were cultured either under serum depletion or at lower concentrations of FBS, the addition of IM to cultures increased the viable cell number, compared with cells cultured without IM (Fig. 1D). All these data suggest a cytoprotective effect in response to IM. It is noteworthy that cytoprotection by IM was pronounced at lower FBS concentrations. Cytoprotection by IM was observed in normal murine embryonic fibroblasts (MEFs) (Supplementary Fig. S1C) and various cancer cell lines (e.g., U937, A549, H266, DLD1, and COLO201) under serum-depleted conditions (data not shown). This result suggests that cytoprotection is ubiquitously induced and is independent of cell type and malignancy of cells.

The cytoprotective effect in response to IM is partially dependent on autophagy induction

It has been reported that IM induces autophagy in various cell lines, although its biological function remains unclear [7]. As autophagy was originally reported as a cell adaptation process under nutrition starvation [6,13], it is enhanced by the depletion of nutrients such as carbon and single amino acids in culture medium [13]. We therefore speculated that cytoprotection in response to IM is mediated through autophagy induction. Electron microscopy demonstrated that treatment with IM increased the number of autophagosomes in HL-60 cells (Fig. 2A). Many intracellular organelles were included in autophagosomes after treatment with IM. However, it has also been reported that ABL kinase regulates the late stage of the autophagy process [14]. Inhibition of ABL kinase reduces the intracellular activities of the lysosomal enzymes, which may result in impaired turnover of long-lived cytosolic proteins and accumulation of autophagosomes [14]. Since ABL is a target of IM as is BCR-ABL, accumulation of autophagosomes in HL-60 cells might be due to inhibition of the flux of autophagy by inhibi-

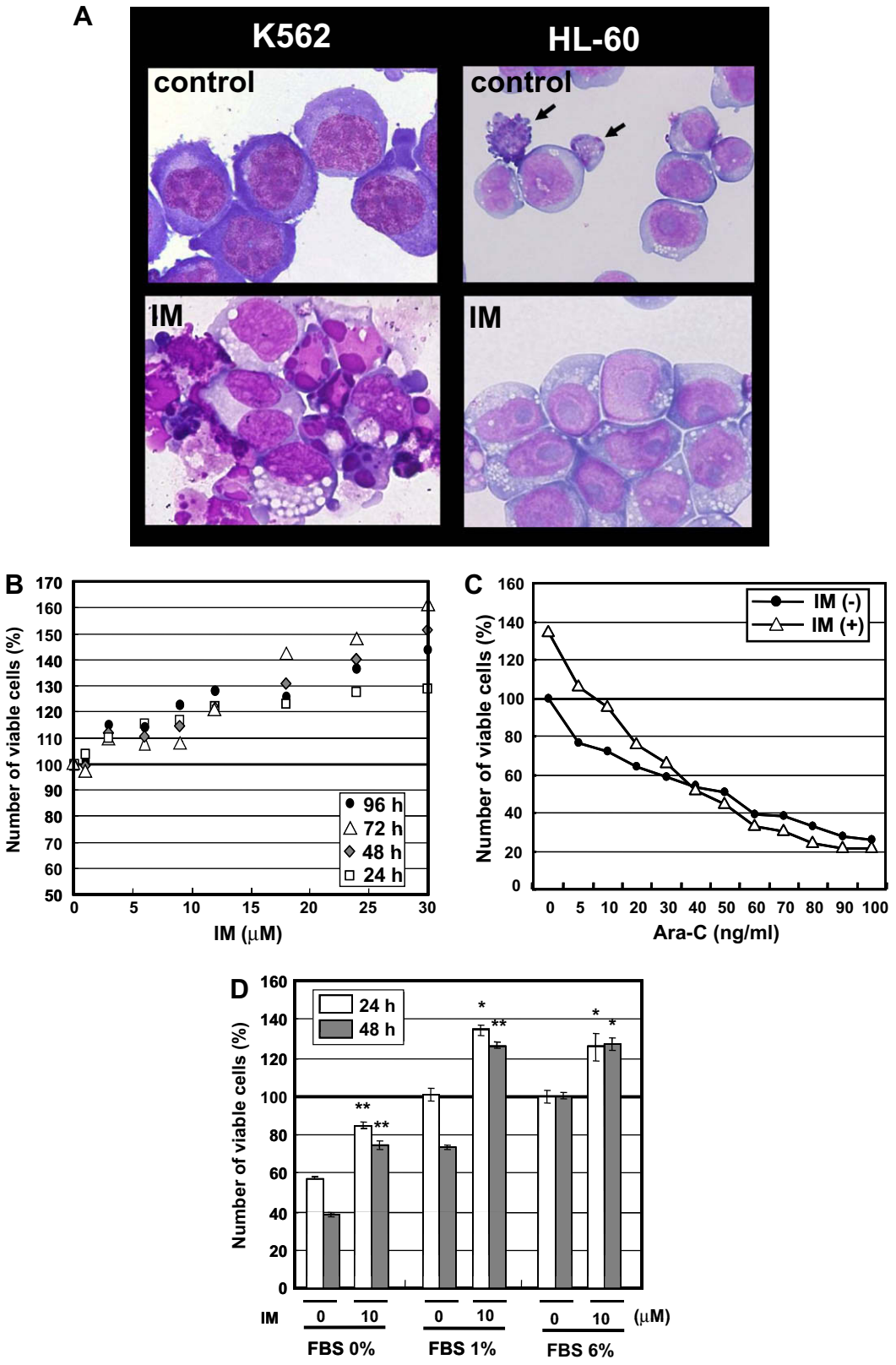

Fig. 1. IM treatment induces a cytoprotective effect in non-BCR-ABL-expressing cells. (A) After 72 h-treatment with 10 μM of IM, HL-60 and K562 cells were processed for May–Grünwald–Giemsa staining to assess morphologic changes (original magnification 1000×). Arrows indicate the HL-60 cells undergoing spontaneous apoptosis in control culture medium. (B) HL-60 cells were cultured with IM at various concentrations for 24–96 h. The relative viable cell numbers compared with the cells without IM treatment are plotted. (C) HL-60 cells were cultured with Ara-C at various concentrations in the presence or absence of 10 μM of IM for 48 h. Viable cell numbers were assessed. (D) HL-60 cells were cultured under FBS-deprived conditions in the presence or absence of IM (10 μM) for 24 and 48 h. Viable cell numbers were assessed. Means ± SD $^{*}p < 0.05$, $^{**}p < 0.01$ vs the cells cultured without IM.

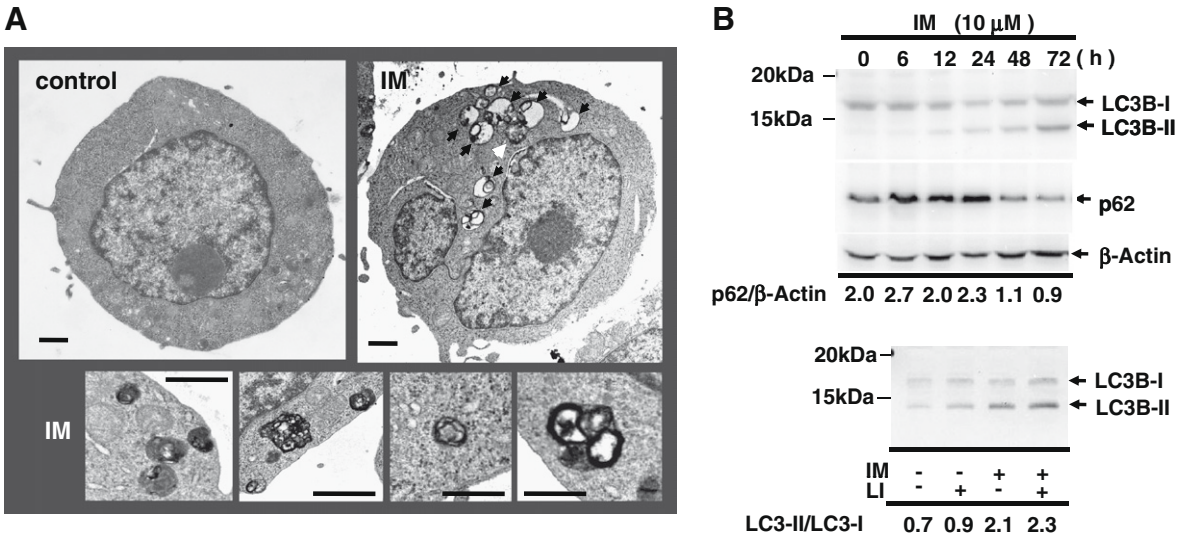

Fig. 2. Autophagy induction after treatment with IM. (A) Ultrastructural features of HL-60 cells by electron microscopy. HL-60 cells were treated with or without IM (10 μM) treatment for 48 h. Arrows indicate autophagosomes. (B) Expression of LC3B isoforms and p62 after treatment with IM in HL-60 cells. Upper panel: after treatment with 10 μM of IM for 6–72 h, cellular proteins were separated by 11.25% SDS–PAGE for LC3B and 15% SDS–PAGE for p62 and immunoblotted with each Ab. The anti-β-actin mAb was used for protein-loading equivalence and the density ratios of p62 to β-actin were analyzed. Lower panel: after 42 h-exposure to 10 μM of IM, HL-60 cells were further cultured with or without lysosomal protease inhibitors (LIs), E-64-d (10 μg/ml) and pepstatin A (10 μg/ml) in the presence of IM for 6 h. Cellular proteins were lysed, separated by 15% SDS–PAGE, and immunoblotted with anti-LC3B Ab. The density ratios of LC3B-II to LC3B-I were analyzed.

tion of ABL kinase as previously reported [14]. Therefore, we next investigated whether or not IM induces autophagy. LC3 protein is known to exist in two cellular forms, LC3-I and LC3-II. LC3-I is converted to LC3-II by conjugation to phosphatidylethanolamine during formation of autophagosome, and the amount of LC3-II is a good early marker for the formation of autophagosomes [15]. Immunoblotting with LC3B Ab demonstrated that IM treatment enhanced LC3B-II formation within 12 h after treatment with IM (Fig. 2B). LC3B-II induction by IM was more potent than those by rapamycin and trehalose, which are well-known autophagy inducers (data not shown) [16,17]. Along with LC3B-II formation, the cellular expression level of p62 (sequestosome-1) was significantly reduced. P62 was originally identified as a component of pathological protein inclusion, but it has now been identified as an adaptor protein with multiple protein–protein interaction domains, including an LC3 interaction region (LIR) for binding the autophagy protein LC3 and a ubiquitin-associated (UBA) domain for ubiquitinated cargo binding [18,19]. Therefore, reduction of p62 expression indicates enhanced degradation via LC3-p62 interaction during autophagy. Furthermore, in the presence of pepstatin A and E-64-d, which are lysosomal protease inhibitors for blocking autophagosome flux, induction of LC3B-II after treatment with IM was further enhanced, compared with those treated with lysosomal inhibitors alone (Fig. 2B and Supplementary Fig. S2) [15]. These data demonstrate that autophagy is enhanced in response to IM in various kinds of cells and that an increased number of autophagosomes is not caused by blocking the autophagy flux alone.

We next examined whether or not cytoprotection in response to IM was dependent on autophagy. We used the conditional knockdown system of Tet-off *atg5* m5–7 cells to completely inhibit the autophagic process [11]. Pre-treatment with doxycycline for 4 days completely inhibited autophagy induction in response to IM by knockdown of Atg5 protein. Under serum-depleted conditions, cytoprotection by IM was significantly suppressed in Atg5 knockdown-m5–7 cells (Fig. 3). This result indicates that cytoprotection is partially dependent on autophagy induction, but other underlying cellular mechanism(s) might exist. To explain autophagy-dependent cytoprotection in response to IM, we hypothesized that the effect is due to enhanced clearance of mitochondria and/or ribosome via autophagy. Selective clearance of mitochondria via autophagy (designated as "mitophagy") may function as reduction

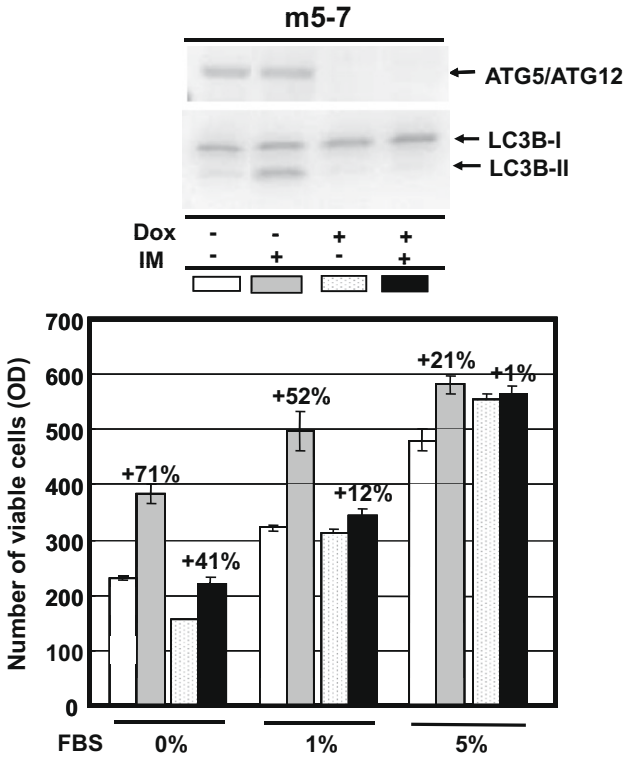

Fig. 3. Induction of cytoprotective autophagy by IM. To knockdown *atg5* gene, the m5–7 cell line was used as a Tet-off atg5 MEF system [17]. After pre-treatment with or without doxycycline (Dox: 10 ng/ml) for 4 days, m5–7 cells were further cultured in the presence or absence of 10 μM of IM for 72 h. Cellular proteins were separated by 11.25% SDS–PAGE and immunoblotted with anti-ATG5 and anti-LC3B Abs (upper panel). Viable cell number of m5–7 cells was assessed using a CellTiter Blue kit. Percentages comparing to the viable cell number without IM treatment were described (lower panel).

of ROS production in the quality control of mitochondria [20], whereas the clearance of ribosome via autophagy (designated as "ribophagy" or "ER-phagy") may function as suppression or regulation of ER stress [21,22]. However, treatment with *N*-acetylcysteine, a scavenger of ROS, did not indicate any cytoprotection in HL-60 cells (Supplementary Fig. S3A). Additionally, we could not detect significant reduction of intracellular total mitochondrial volume or mitochondrial superoxide production after treatment of HL-60 cells with IM (Supplementary Fig. S3B). Next, we examined whether or not IM reduces ER stress. Treatment of the cells with tunicamycin (TN), which inhibits the synthesis of all N-linked glycoproteins, causes ER stress. In the series of unfolded protein responses (UPRs), TN treatment induces up-regulation of CHOP (GADD153) expression followed by apoptosis induction [23]. TN treatment inhibited cell growth in HL-60 cells, whereas combined treatment with IM and TN attenuated TN-induced cell-growth inhibition (Supplementary Fig. S3C). This result suggests that the reduction of ER stress via ribophagy might be involved. However, although treatment with TN induced up-regulation of CHOP in our system, the combined treatment of IM plus TN did not induce reduction; instead, it enhanced CHOP expression, compared with treatment by TN alone (Supplementary Fig. S3D). This result indicates that IM does not suppress TN-induced ER stress. Enhanced expression of CHOP after combined treatment with IM plus TN might be explained in part by the blocking of autophagy flux by inhibiting ABL kinase activity, as previously reported [14]. However, this is inconsistent with a recent report demonstrating that IM reduced ER stress and induced remission of diabetes in db/db diabetic model mice [24]. The contrasting effects of IM on ER stress may be due to experiment conditions such as *in vitro* vs *in vivo* or to a difference of cell types. Further studies are required.

Since activation of ABL kinase has been reported to induce apoptosis [25], cytoprotection by IM may partially be due to inhibition of ABL. Here, cytoprotection by IM may be explained by two independent mechanisms, cytoprotective autophagy induction and ABL kinase inhibition. We next tried to induce gene silence of *c-abl* in HL-60 cells using *c-abl* shRNA lentiviral particles. After knockdown of ABL expression, HL-60 cells still exhibited IM-induced cytoprotection under serum deprivation. In addition, attenuation of Ara-C-induced cytotoxicity was still detectable (Supplementary Fig. S4). This result suggests that cytoprotection does not depend on ABL kinase activity.

All these data demonstrate that IM exerts a cytoprotective effect in various kinds of cells. This effect at least partially depends on autophagy induction, but is not mediated through inhibition of ROS production via mitochondrial clearance or reduction of ER stress via ribosomal clearance. Not demonstrated here is the induction of cytoprotection after the cells were treated with other tyrosine kinase inhibitors such as nilotinib and dasatinib but not with sunitinib. Therefore, inhibition of some tyrosine kinase(s) appears to be involved in this scenario. The cytoprotective effect by IM exhibited here may explain the reported therapeutic effect of IM in diabetic model mice [9]. Furthermore, this cytoprotective effect provides clinical possibilities, including *ex vivo* stem-cell expansion. Identification of the target tyrosine kinase(s) as the next step is warranted.

Acknowledgments

We thank Dr. Noboru Mizushima (Tokyo Medical and Dental University School of Health Science, Tokyo, Japan) for a kind gift of Tet-off *atg5* m5–7 cells. We also thank Novartis Pharma AG (Basel, Switzerland) for providing IM, and Nippon Shinyaku Co., Ltd. (Kyoto, Japan) for providing Ara-C. This study is supported in part by funds from the Private University Strategic Research-Based Support Project (Molecular Information-based Intractable Disease Research Project) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (2008-2012) to A.T. and K.M., and by a grant from the Tokyo Medical University Cancer Research Foundation to K.M.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.11.055.

References

[1] B.J. Druker, S. Tamura, E. Buchdunger, S. Ohno, G.M. Segal, S. Fanning, J. Zimmermann, N.B. Lydon, Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells, Nat. Med. 2 (1996) 561–566.
[2] G.D. Demetri, M. von Mehren, C.D. Blanke, A.D. Van den Abbeele, B. Eisenberg, P.J. Roberts, M.C. Heinrich, D.A. Tuveson, S. Singer, M. Janicek, J.A. Fletcher, S.G. Silverman, S.L. Silberman, R. Capdeville, B. Kiese, B. Peng, S. Dimitrijevic, B.J. Druke, C. Corless, C.D. Fletcher, H. Joensuu, Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors, N. Engl. J. Med. 347 (2002) 472–480.
[3] J. Cools, D.J. DeAngelo, J. Gotlib, E.H. Stover, R.D. Legare, J. Cortes, J. Kutok, J. Clark, I. Galinsky, J.D. Griffin, N.C. Cross, A. Tefferi, J. Malone, R. Alam, S.L. Schrier, J. Schmid, M. Rose, P. Vandenberghe, G. Verhoef, M. Boogaerts, I. Wlodarska, H. Kantarjian, P. Marynen, S.E. Coutre, R. Stone, D.G. Gilliland, A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome, N. Engl. J. Med. 348 (2003) 1201–1214.
[4] B.J. Druker, F. Guilhot, S.G. O'Brien, I. Gathmann, H. Kantarjian, N. Gattermann, M.W. Deininger, R.T. Silver, J.M. Goldman, R.M. Stone, F. Cervantes, A. Hochhaus, B.L. Powell, J.L. Gabrilove, P. Rousselot, J. Reiffers, J.J. Cornelissen, T. Hughes, H. Agis, T. Fischer, G. Verhoef, J. Shepherd, G. Saglio, A. Gratwohl, J.L. Nielsen, J.P. Radich, B. Simonsson, K. Taylor, M. Baccarani, C. So, L. Letvak, R.A. Larson, IRIS Investigator, five-year follow-up of patients receiving imatinib for chronic myeloid leukemia, N. Engl. J. Med. 355 (2006) 2408–2417.
[5] B. Levine, G. Kroemer, Autophagy in the pathogenesis of disease, Cell 132 (2008) 27–42.
[6] N. Mizushima, Physiological functions of autophagy, Curr. Top. Microbiol. Immunol. 335 (2009) 71–84.
[7] A. Ertmer, V. Huber, S. Gilch, T. Yoshimori, V. Erfle, J. Duyster, H.P. Elsässer, H.M. Schätzl, The anticancer drug imatinib induces cellular autophagy, Leukemia 21 (2007) 936–942.
[8] C. Bellodi, M.R. Lidonnici, A. Hamilton, G.V. Helgason, A.R. Soliera, M. Ronchetti, S. Galavotti, K.W. Young, T. Selmi, R. Yacobi, R.A. Van Etten, N. Donato, A. Hunter, D. Dinsdale, E. Tirrò, P. Vigneri, P. Nicotera, M.J. Dyer, T. Holyoake, P. Salomoni, B. Calabretta, Targeting autophagy potentiates tyrosine kinase inhibitor-induced cell death in Philadelphia chromosome-positive cells, including primary CML stem cells, J. Clin. Invest. 119 (2009) 1109–1123.
[9] C. Louvet, G.L. Szot, J. Lang, M.R. Lee, N. Martinier, G. Bollag, S. Zhu, A. Weiss, J.A. Bluestone, Tyrosine kinase inhibitors reverse type 1 diabetes in nonobese diabetic mice, Proc. Natl. Acad. Sci. USA 105 (2008) 18895–18900.
[10] T. Yokoyama, K. Miyazawa, M. Naito, J. Toyotake, T. Tauchi, M. Itoh, A. Yuo, Y. Hayashi, M.N. Georgescu, Y. Kondo, S. Kondo, K. Ohyashiki, Vitamin K2 induces autophagy and apoptosis simultaneously in leukemia cells, Autophagy 4 (2008) 629–640.
[11] N. Hosokawa, Y. Hara, N. Mizushima, Generation of cell lines with tetracycline-regulated autophagy and a role for autophagy in controlling cell size, FEBS Lett. 580 (2006) 2623–2639.
[12] G. Fang, C.N. Kim, C.L. Perkins, N. Ramadevi, E. Winton, S. Wittmann, K.N. Bhalla, CGP57148B (STI-571) induces differentiation and apoptosis and sensitizes Bcr-Abl-positive human leukemia cells to apoptosis due to antileukemic drugs, Blood 96 (2000) 2246–2253.
[13] K. Takeshige, M. Baba, S. Tsuboi, T. Noda, Y. Ohsumi, Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction, J. Cell. Biol. 119 (1992) 301–311.
[14] G. Togalingam, M. Pnedergast, Abl kinases regulates autophagy by promoting the trafficking and function of lysosomal components, J. Biol. Chem. 283 (2008) 35941–35953.
[15] N. Mizushima, T. Yoshimori, How to interpret LC3 immunoblotting, Autophagy 3 (2007) 542–545.
[16] S. Sarkar, J.E. Davies, Z. Huang, A. Tunnacliffe, D.C. Rubinsztein, Trehalose, a novel mTOR-independent autophagy enhancer, accelerates the clearance of mutant huntingtin and alpha-synuclein, J. Biol. Chem. 282 (2007) 5641–5652.
[17] T. Pan, P. Rawal, Y. Wu, W. Xie, J. Jankovic, W. Le, Rapamycin protects against rotenone-induced apoptosis through autophagy induction, Neuroscience (2009) (Epub ahead of print).
[18] J. Moscat, M.T. Diaz-Meco, P62 at the crossroads of autophagy, apoptosis, and cancer, Cell 137 (2009) 1001–1004.
[19] V. Kirkin, D.G. McEwan, I. Novak, I. Dikic, A role for ubiquitin in selective autophagy, Mol. Cell 34 (2009) 259–269.
[20] H. Abeliovich, Mitophagy: the life-or-death dichotomy includes yeast, Autophagy 3 (2007) 275–277.

[21] C. Kraft, A. Deplazes, M. Sohrmann, M. Peter, Mature ribosomes are selectively degraded upon starvation by an autophagy pathway requiring the Ubp3p/Bre5p ubiquitin protease, Nat. Cell Biol. 10 (2008) 602–610.
[22] S. Bernales, S. Schuck, P. Walter, ER-phagy: selective autophagy of the endoplasmic reticulum, Autophagy 3 (2007) 285–287.
[23] H. Zinszner, M. Kuroda, X. Wang, N. Batchvarova, R.T. Lightfoot, H. Remotti, J.L. Stevens, D. Ron, CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum, Genes Dev. 12 (1998) 982–995.
[24] M.S. Han, K.W. Chung, H.G. Cheon, S.D. Rhee, C.H. Yoon, M.K. Lee, K.W. Kim, M.S. Lee, Imatinib mesylate reduces endoplasmic reticulum stress and induces remission of diabetes in db/db mice, Diabetes 58 (2009) 329–336.
[25] J.Y.J. Wang, Regulation of cell death by the Abl tyrosine kinase, Oncogene 19 (2000) 5643–5650.