Biochemical and Biophysical Research Communications 429 (2012) 87–92

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

ETV6–NTRK3 as a therapeutic target of small molecule inhibitor PKC412

Hoang Thanh Chi [a,*], Bui Thi Kim Ly [a], Yasuhiko Kano [b], Arinobu Tojo [c], Toshiki Watanabe [a], Yuko Sato [d]

[a] Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Tokyo 108-8639, Japan
[b] Division of Hematology and Medical Oncology, Tochigi Cancer Center, Tochigi 321-0293, Japan
[c] Division of Molecular Therapy, Department of Hematology/Oncology, Research Hospital, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan
[d] Musashimurayama Hospital, Musashimurayama, Tokyo 208-0011, Japan

ARTICLE INFO

Article history:
Received 20 October 2012
Available online 3 November 2012

Keywords:
AML
ETV6–NTRK3
PKC412
IMS-M2
M0-91

ABSTRACT

The ETV6–NTRK3 (EN) fusion gene which encodes a chimeric tyrosine kinase was first identified by cloning of the t(12;15)(p13;q25) translocation in congenital fibrosarcoma (CFS). Since then, EN has been also found in congenital mesoblastic nephroma (CMN), secretory breast carcinoma (SBC) and acute myelogenous leukemia (AML). Using IMS-M2 and M0–91 cell lines harboring the EN fusion gene, and Ba/F3 cells stably transfected with EN, we demonstrated that PKC412, also known as midostaurin, is an inhibitor of EN. Inhibition of EN activity by PKC412 suppressed the activity of it downstream molecules leading to inhibition of cell proliferation and induction of apoptosis. Our data for the first time suggested that PKC412 could serve as therapeutic drug for treatment of patients with this fusion.

1. Introduction

The ETV6–NTRK3 (EN) fusion gene which encodes a chimeric tyrosine kinase was first identified by cloning of the t(12;15)(p13;q25) translocation in congenital (or infantile) fibrosarcoma, a mesenchymal malignancy of very young children [1]. Since then, EN has been also found in congenital mesoblastic nephroma [2], secretory breast carcinoma [3] and acute myelogenous leukemia (AML) [4].

ETV6 (also known as *TEL*) is an ETS family transcription factor thought to play a major role in early hematopoiesis and angiogenesis [5,6]. The *ETV6* gene has also been identified as a fusion partner in leukemia-associated chimeric proteins, such as *ETV6-PDGFR* [7], *ETV6-AML1* [8,9], *ETV6-JAK2* [10], *ETV6-ARG* [11], and others [12]. The *NTRK3* gene (also known as *TRKC*) encodes the transmembrane surface receptor for neurotrophin-3 involved in growth, development, and cell survival in the central nervous system [13].

In general, EN fusion transcripts encode the N-terminal pointed (PNT) domain of *ETV6* which is responsible for polymerization [14] fused to the C-terminal protein tyrosine kinase (PTK) domain of NTRK3. This fusion protein is similar in structure to other ETV6 chimeric PTKs [1]. Until now, two types of this fusion gene were found. The first one is detected in the non-hematological maglinancies in which the chimeric transcript encoded exon 1 to exon 5 of the *ETV6* gene fused to nucleotide (nt) 1741 of *NTRK3* gene. The second one is detected in leukemia in which the chimeric transcript encoded exon 1 to exon 4 of *ETV6* gene fused to nt 1741 of *NTRK3* gene.

In general, native *NTRK3* requires an extracellular ligand binding of neurotrophin 3 prior to its dimerization and autophosphorylation [15–19]. However, when fused to ETV6, the extracellular ligand binding domain of NTRK3 is abrogated and ETV6–NTRK3 bypassed this requirement, still keep itself autophosphorylation. Interestingly, in vitro and in vivo experiments have shown that the EN fusion protein has potential transforming activity in several cell lineages including fibroblast [20], hematopoietic cells [21], and breast epithelial cells [3].

The important role of EN in oncogenesis has been well known. However, specific treatment for patients expressing EN has not been achieved. Patients of congenital fibrosarcoma, congenital mesoblastic nephroma and secretory breast carcinoma have been considered as a good prognosis and rarely metastases [22–24]. Unfortunately, patients of leukemia seem to be poor prognosis. Two patients harboring EN have been reported no response to chemotherapy treatment suggesting it as a refractory leukemia [25,26]. Therefore, finding novel therapeutic treatment for leukemia patients harboring EN is extremely necessary.

We hypothesized that inhibition of EN could be an effective therapeutic strategy in treatment of patients harboring EN. In this report, we tested the potential therapeutic utility of the small molecule inhibitor PKC412 as an option for treatment of leukemias associated with EN fusion. PKC412 also known as midostaurin is the broad spectrum inhibitor of serine-threonine/tyrosine-protein kinases including protein kinase C (PKC), vascular endothelial growth factor receptor (VEGFR), fms-like tyrosine kinase (FLT3),

* Corresponding author. Address: Department of Medical Genome Sciences, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan. Fax: +81 3 5449 5298.
E-mail address: kk086406@mgs.k.u-tokyo.ac.jp (H.T. Chi).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.10.087

platelet-derived growth factor receptor (PDGFR) and the stem cell factor, c-KIT [27,28]. Currently, PKC412 is used in phase IIB clinical trials for treatment of acute myeloblastic leukemia (AML) patients with FTL3 mutations [29] with minimal side-effect suggesting the utility of PKC412 for treatment.

2. Materials and methods

2.1. Plasmid construction

The construct of AML type of EN was described somewhere [30].

2.2. Cell lines, culture conditions and transfection

Experiments were conducted using two EN-positive AML cell lines: IMS-M2 and M0–91. IMS-M2 cell line was established from the bone marrow cells taken from a 59-year-old female with AML (FAB-M2), with chromosome abnormalities of 48,XX,add(6)(q27),+8, der(12)t(12;15)(p13;q25)inv(12)(p13;q15),der(15) t(12;15)(p13;q25), +der(15)t(12;15)(p13;q25), ETV6–NTRK3 fusion gene [4,25] and the type A *NPM1* mutation [31]. M0–91, an AML-M0 derived cell line, has recently been identified as a cell line expressing the ETV6–NTRK3 fusion gene by Gu et al. [32]. MOLM-13 and Jurkat cells were used as controls for evaluating PKC412 anti-proliferation effect.

All cell lines were grown in RPMI 1640 medium (Sigma–Aldrich, Japan K.K., Tokyo, Japan) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (JRH Biosciences, Lenexa, KS, USA), 100 IU/ml penicillin, and 0.1 mg/ml streptomycin (Nakalai Tesque, Kyoto, Japan) in a humidified incubator of 5% CO_2 at 37 °C. The Ba/F3 cells (ATCC, Manassas, VA, USA), maintained in RPMI 1640 medium supplemented with 10% FBS and 10 ng/ml of recombinant mouse interleukin 3 (IL-3; R&D Systems, Minneapolis, MN) were transfected with AML type of EN construct by lipofectamine (Invitrogen) according to the manufacturer's instructions. Transfected cells were selected in medium containing mouse IL-3 and 1 mg/ml G418 (GIBCO BRL, Gaithersburg, MD, USA) for 2 weeks and subsequently subjected to limiting dilution to isolate single clones.

2.3. Reagents

PKC412, midostaurin was purchased from Sigma–Aldrich Japan (Tokyo, Japan) and dissolved in dimethylsulfoxide (DMSO). Controlled cells were cultured with the same concentration of carrier DMSO as used in the highest dose of reagents. The concentration of DMSO was kept under 0.1% throughout all the experiments to avoid its cytotoxicity.

2.4. Cell proliferation assays

Proliferation was determined by trypan blue dye exclusion test. Cells in suspension were seeded in six-well plates at a density of 1×10^5 cells/ml in the presence of different concentrations of PKC412 for 3 days. In control wells, DMSO instead of PKC412 was added. After the treatment, 10 μl of the cell suspension was mixed with 10 μl of 0.4% trypan blue, and alive cells were counted manually using a hemacytometer. Results were calculated as the percentage of the values measured when cells were grown in the absence of the reagent. All experiments were performed in triplicate.

2.5. Western Blot analysis

The western Blot analysis was described in previous report [33]. Immunoprecipitation (IP) was performed as described previously [34]. Protein samples were electrophoresed through polyacrylamide gel and transferred to Hypond-P membrane (Amersham, Buckinghamshire, UK) by electro-blotting. After washing, the membrane was probed with following antibodies and antibody-binding was detected using enhanced chemiluminescence (ECL) (Amersham). The following antibodies were purchased from Cell Signaling Technology Japan (Tokyo, Japan): Phospho-p44/42 Map kinase (Thr202/Tyr204), phospho-Akt (Ser473), XIAP, Bcl-2, caspase-3, PARP, AKT, p44/42 MAPK, phospho-IKappaBalpha (Ser 32/36), and p-STAT5 (Y694). α-TrkC (C-14), STAT5 (C-17), and NFkB p52 (C-5), survivin (sc-17779), anti-rabbit IgG-HRP (sc-2317), and anti-mouse IgG-HRP (sc-2031) antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-phosphotyrosine 4G10 was from Upstate Biotechnology (Lake Placid, NY, USA). Anti-actin (A2066) was from Sigma–Aldrich.

2.6. Wright-Giemsa staining

For fragmented nuclei and condensed chromatin assessment, cells at a density of 1×10^5 cells/ml were treated with 100 nM PKC412. After 24 h incubation, cells were harvested and fixed onto slides by using a cytospin (Shandon, Shandon Southern Products Ltd., Cheshire, UK). Cells then were stained with Wright-Giemsa solution. Morphology of cells was observed under an inverted microscope.

2.7. DNA fragmentation assay

IMS-M2 cells were treated with or without 100 nM PKC412 for 24 h. Cells then were collected and total genomic DNA (gDNA) was extracted with a standard protocol. For DNA fragmentation assay, 10 μg gDNA of each sample was blotted and electrophoresed on 1.2% agarose gel. DNA fragmentation was observed under UV light.

2.8. Statistical analysis

All data were expressed as the mean ± standard deviation. Statistical analyses were done using Student's *t-test*, in which $p < 0.05$ was the minimum requirement for a statistically significant difference.

3. Results

3.1. PKC412 inhibits EN fusion tyrosine kinase in hematopoietic Ba/F3 cells

We evaluated the transforming property of EN in hematopoietic Ba/F3 cells. EN construct was stably transduced in Ba/F3 cells (Fig. 1A). Stable Ba/F3 cell line was assessed for IL-3 independent growth as a surrogate for transformation (Fig. 1B). EN effectively conferred IL-3 independence to Ba/F3 cells, whereas, Ba/F3 cells transduced with empty vector underwent apoptosis in the absence of IL-3.

To determine whether PKC412 inhibited EN activity, we have used the assay based on the work of Daley and Baltmore [35]. The transfected Ba/F3 cells were treated with or without 100 nM PKC412 for 72 h. Then cell proliferation assay was done to account for the inhibitory effect of reagent. 100 nM PKC412 significantly inhibited the cell proliferation of transfected Ba/F3 cells (Fig. 1C). To confirm whether the cell growth inhibition of PKC412 in transfected Ba/F3 cells is due to the lost of EN phosphorylation, we then checked the phosphorylation of EN in transfected Ba/F3 cells treated with or without 100 nM PKC412 for 8 h. As expected, the phosphorylation of this fusion protein was decreased by PKC412 treatment (Fig. 1D), suggesting that PKC412 inhibited the cell pro-

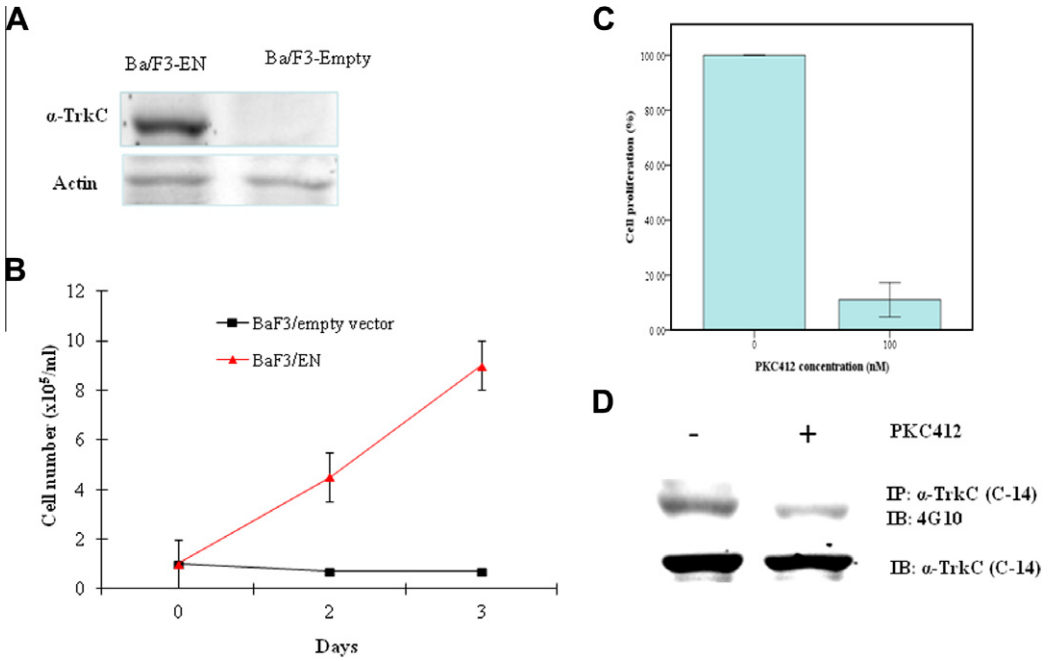

Fig. 1. PKC412 inhibits EN fusion tyrosine kinase in hematopoietic Ba/F3 cells. EN confers IL-3 independent growth to Ba/F3 cells. Successful transduction of EN construct (A) into Ba/F3 cells could induce IL-3 independent growth of Ba/F3 cells (B). 100 nM PKC treatment strongly inhibited the cell proliferation of EN-transfected Ba/F3 cells (C) and the activation of EN (D).

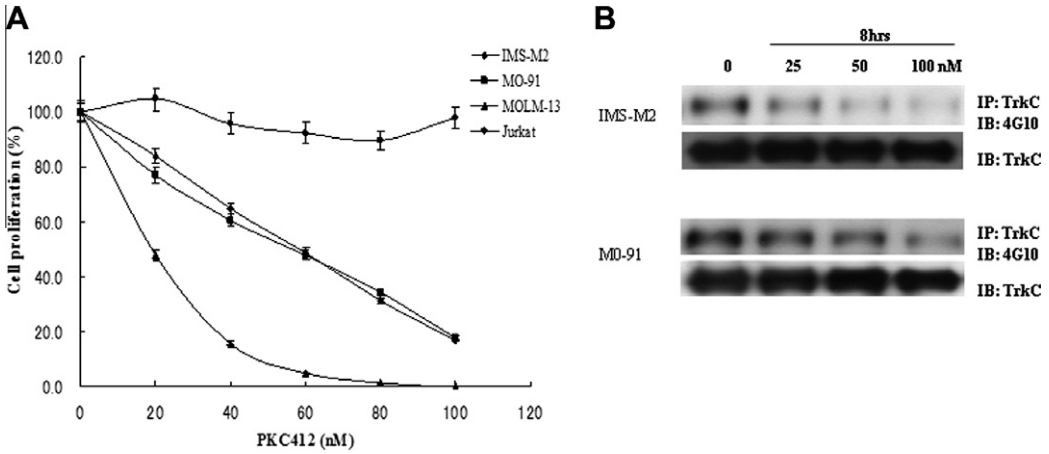

Fig. 2. Growth inhibition of EN-positive cell lines by PKC412. Panel A showed the anti-proliferation effect of PKC412 in cell lines harboring EN. Cell lines including IMS-M2, M0–91, MOLM-13 and Jurkat at a density of 1×10^5 cells/ml were treated with 10, 20, 40, 60, 80, 100 nM PKC412 or DMSO alone (0 nM PKC412) as control for 72 h. The number of alive cells was counted after trypan blue exclusion test. Results were calculated as the percentage of the control values. Panel B showed the effect of PKC412 on inhibition of EN activity in M0–91 and IMS-M2 cells after 8 h treatment.

liferation of transfected Ba/F3 cells by inhibiting the phosphorylation of EN. Altogether, the data demonstrate that EN could be a target of PKC412.

3.2. Growth inhibition of EN-positive cell lines by PKC412

Next we tested the inhibitory effect of PKC412 in a more clinically relevant system by performing a dose–response analysis using EN-positive cell lines including IMS-M2 and M0–91. Cell proliferation was evaluated using the trypan blue exclusion test and was confirmed by WST assay [36]. The growth of IMS-M2 and M0–91 cells was significantly inhibited in a dose-dependent manner by PKC412 treatment (Fig. 2A). However, the inhibitory effect of PKC412 obtained in MOLM-13 cells is more sensitive than in EN-positive cells. In contrast, the proliferation of EN-negative Jurkat cells seems to be not affected even at 100 nM PKC412 treatment (Fig. 2A).

To confirm whether PKC412 inhibited EN activity in EN-positive cell lines, we checked the phosphorylation status of EN in both two cell lines after 8 h treated with different concentration of PKC412. The results showed that PKC412 significantly inhibited EN phosphorylation in M0–91 and IMS-M2 cells in a dose-dependent manner (Fig. 2B). The data confirmed that EN is a target of PKC412.

3.3. Effect of PKC412 on AKT, MAPK, and STAT5 phosphorylation

To check whether PKC412 inhibits phosphorylation of *AKT*, *MAPK* and *STAT5*, we measure the phosphorylation status of these molecules after treated with 100 nM PKC412. The phosphorylation of the AKT, MAPK and STAT5 was significantly inhibited by PKC412

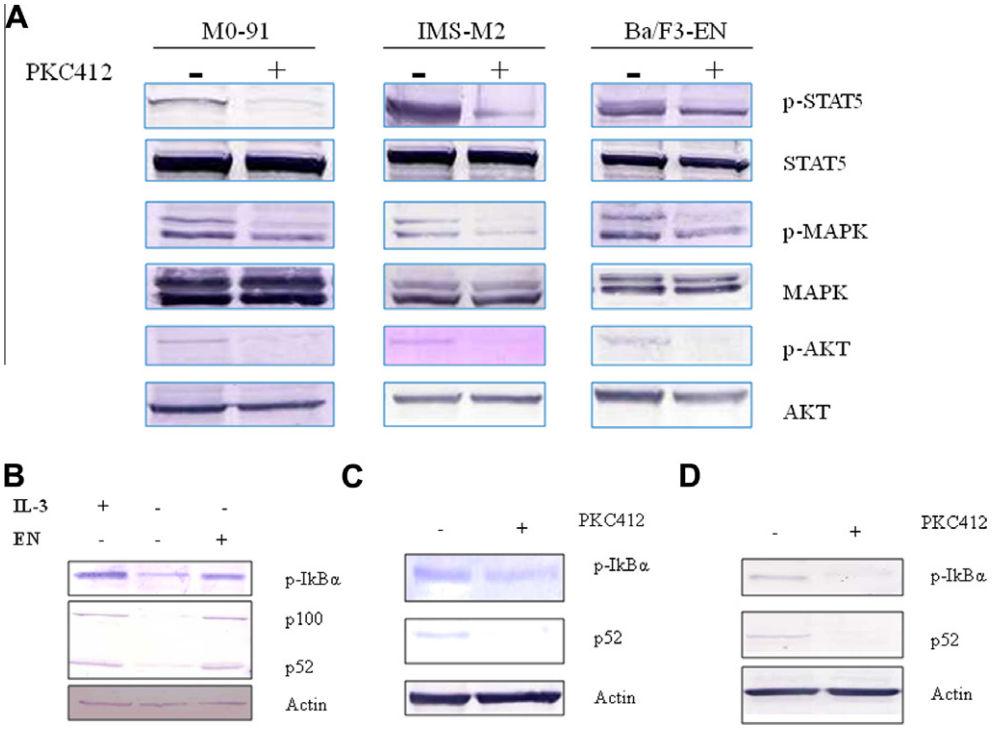

Fig. 3. Effect of PKC412 on EN-mediated signaling pathway Cells expressing EN have been treated with 100 nM PKC412 for 8 h. Total cell lysates were prepared and subjected to western blot analysis. Panel A showed that p-STAT5, p-AKT and p-MAPK were suppressed by PKC412 treatment. Panel B showed that EN could induce the activation of NF-κB pathway in EN-transfected Ba/F3 cells. PKC412 treatment could suppress the activation of p-IκBα and p52 in transfected Ba/F3 (C) and M0–91 cells (D).

(Fig. 3A). These results are consistent with previous reports that EN is the upstream molecule of Ras-MAPK and PI3K-AKT pathways [37].

3.4. PKC412 suppresses the activity of NFB

The EN-mediated signaling pathways controlling the cell proliferation and survival is not fully understood. In this study, we have shown that EN could induce the activation of NF*κB*. Introduction of EN construct into the Ba/F3 cells increases the phosphorylation of IκBα and the activation of p52 (Fig. 3B).

To test whether PKC412 treatment could affect on the activity of NF*κB*, M0–91 and Ba/F3 expressing EN cells were treated with PKC412 and subjected to western blot analysis. PKC412 treatment suppressed the phosphorylation of IκBα and the expression of p.52 in both EN-transfected Ba/F3 (Fig. 3C) and M0–91 (Fig. 3D) cells. In addition, treatment with DHMEQ, an inhibitor of NF*κB*, could suppress the proliferation of IMS-M2, M0–91 and EN-transfected Ba/F3 cells (data not shown). It suggests that NF*κB* pathway might also play an essential role in EN-inducing transformation.

3.5. PKC412 induces apoptosis in EN-positive cells

To determine whether PKC412 induces apoptosis in these cells, we have checked the appearance of some apoptotic markers in IMS-M2 after PKC412 treatment by western blot. As a result, 100 nM PKC412 treatment for 24 h induced the activation of caspase-3 (Fig. 4A, the top row) and subsequently inactive the activity of PARP (Fig. 4A, the bottom row). Moreover, PKC412 treatment showed the inhibition of survivin, XIAP and Bcl-2 expression (Fig. 4B). The PKC412-induced apoptosis is further supported by the morphology findings that PKC412 treatment show the DNA damage (Fig. 4C) and appearance of apoptotic bodies in IMS-M2 cells (Fig. 4D). Overall, the PKC412-induced cell death in IMS-M2 is apoptosis.

4. Discussion

Small molecule tyrosine kinase inhibitors, such as imatinib (Gleevec) or gefitinib (Iressa), are efficacious in treating certain human malignancies and solid tumors associated with dysregulation of tyrosine kinases. For example, imatinib has been successfully applied in clinical treatments of BCR-ABL-associated chronic myelogenous leukemia (CML), and activating mutations of c-KIT-associated gastrointestinal stromal cell tumors (GIST) [38,39]. In this report, we evaluated PKC412 as an inhibitor of activating EN. We observed that PKC412 effectively inhibits EN-dependent growth of Ba/F3 cells, as well as kinase activity and activation of downstream signaling effectors. Moreover, similar results were obtained in inhibition of EN-positive human cell lines by PKC412. The sensitivity to PKC412 is different among cell lines. MOLM-13 cells which harboring FLT-3 mutation showed more sensitive to PKC412 than EN-positive cell lines (Fig. 2A). It has been reported that PKC412 inhibits the growth of cancer cells with IC_{50} from 200–700 nM [40] comparing with IC_{50} obtained in EN-positive cell lines (60 nM) indicating that PKC412 could be a potential therapeutic reagent for treating this kind of cancers. However, in vivo experiments need to be done to test the effect of PKC412 on animal model.

The strength in the utility of PKC412 as a novel small molecule inhibitor in the treatment of EN-associated leukemia is that PKC412 is currently being evaluated in human clinical trials for treatment of AML associated with FLT3 with minimal effects, whereas no other EN inhibitors have been reported in the literature, to our knowledge, to be efficacious and well tolerated in patients. This brings much promise to patients who receive current highly aggressive therapies such as high-dose chemo or radio-ther-

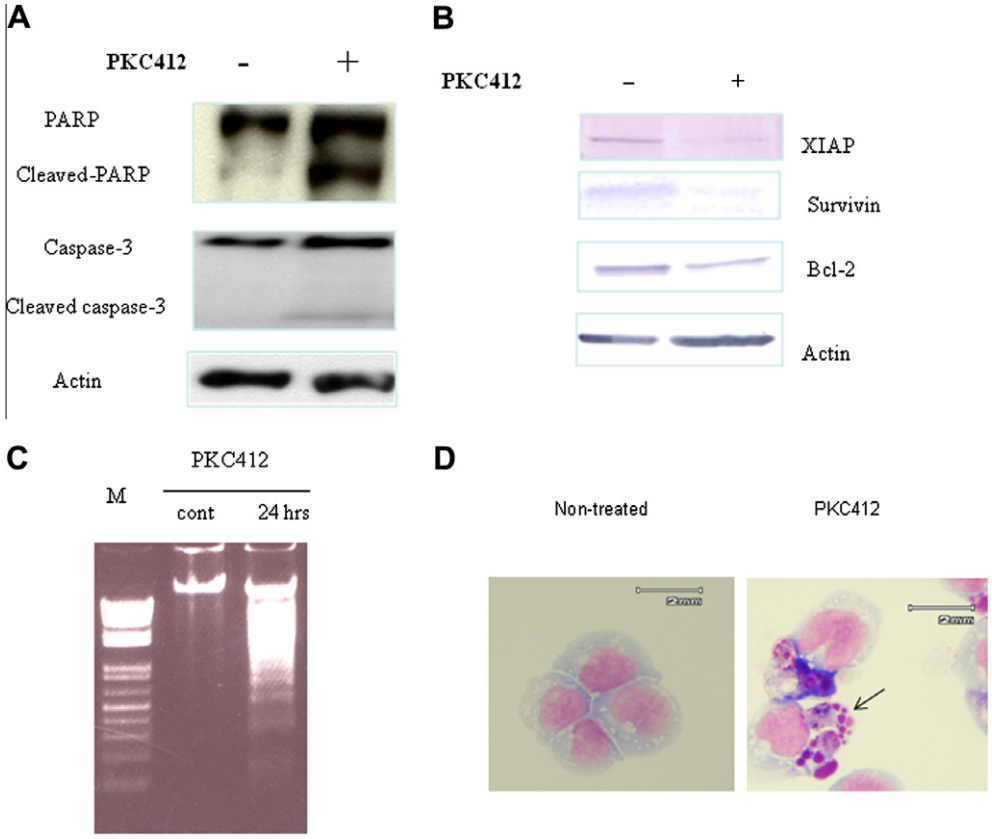

Fig. 4. PKC412 induces apoptosis in EN-positive cells IMS-M2 cells were treated with 100 nM PKC412 for 24 h. Cell lysates were subjected to western blot analysis with caspase-3 and PARP antibody. The results showed that treatment with PKC412 induced the cleavage of caspase-3 and PARP (A). 8 h treatment with PKC412 resulted in suppression of XIAP, survivin and Bcl-2 expression (B). Panel C showed the result of DNA fragmentation and panel D showed the morphological changes of IMS-M2 cells by PKC412 treatment. The arrow showed the appearance of apoptotic body in IMS-M2 cells after 24 h treating with PKC412.

apy followed by autologous transplantation of hematopoietic stem cells.

EN has also been identified in human congenital fibrosarcoma [1], congenital mesoblastic nephroma [2], and secretory breast carcinoma [3]. Therefore, our findings that the small molecule tyrosine inhibitor PKC412 inhibits EN activation may have therapeutic implications in the treatments of not only hematopoietic malignancies but also solid tumors associated with EN fusion.

Acknowledgments

We thank Dr. Mariko Eguchi for providing us the construct of AML type of ETV6–NTRK3 and Dr. Kondo for providing M0-91 cells. This work was supported in part by Global COE Program "Center of Education and Research for the Advanced Genome-Based Medicine – For personalized medicine and the control of worldwide infectious diseases – ", MEXT, Japan.

References

[1] S.R. Knezevich, D.E. McFadden, W. Tao, J.F. Lim, P.H. Sorensen, A novel ETV6–NTRK3 gene fusion in congenital fibrosarcoma, Nat. Genet. 18 (1998) 184–187.

[2] S.R. Knezevich, M.J. Garnett, T.J. Pysher, J.B. Beckwith, P.E. Grundy, P.H. Sorensen, ETV6–NTRK3 gene fusions and trisomy 11 establish a histogenetic link between mesoblastic nephroma and congenital fibrosarcoma, Cancer Research 58 (1998) 5046–5048.

[3] C. Tognon, S.R. Knezevich, D. Huntsman, C.D. Roskelley, N. Melnyk, J.A. Mathers, L. Becker, F. Carneiro, N. MacPherson, D. Horsman, C. Poremba, P.H. Sorensen, Expression of the ETV6–NTRK3 gene fusion as a primary event in human secretory breast carcinoma, Cancer Cell 2 (2002) 367–376.

[4] M. Eguchi, M. Eguchi-Ishimae, A. Tojo, K. Morishita, K. Suzuki, Y. Sato, S. Kudoh, K. Tanaka, M. Setoyama, F. Nagamura, S. Asano, N. Kamada, Fusion of ETV6 to neurotrophin-3 receptor TRKC in acute myeloid leukemia with t(12;15)(p13;q25), Blood 93 (1999) 1355–1363.

[5] M.J. Edel, The ETS-related factor TEL is regulated by angiogenic growth factor VEGF in HUVE-cells, Anticancer Res. 18 (1998) 4505–4509.

[6] L.C. Wang, W. Swat, Y. Fujiwara, L. Davidson, J. Visvader, F. Kuo, F.W. Alt, D.G. Gilliland, T.R. Golub, S.H. Orkin, The TEL/ETV6 gene is required specifically for hematopoiesis in the bone marrow, Genes Development 12 (1998) 2392–2402.

[7] T.R. Golub, G.F. Barker, M. Lovett, D.G. Gilliland, Fusion of PDGF receptor beta to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation, Cell 77 (1994) 307–316.

[8] T.R. Golub, G.F. Barker, S.K. Bohlander, S.W. Hiebert, D.C. Ward, P. Bray-Ward, E. Morgan, S.C. Raimondi, J.D. Rowley, D.G. Gilliland, Fusion of the TEL gene on 12p13 to the AML1 gene on 21q22 in acute lymphoblastic leukemia, Proc. Natl. Acad. Sci. USA 92 (1995) 4917–4921.

[9] S.P. Romana, M. Mauchauffe, M. Le Coniat, I. Chumakov, D. Le Paslier, R. Berger, O.A. Bernard, The t(12;21) of acute lymphoblastic leukemia results in a tel-AML1 gene fusion, Blood 85 (1995) 3662–3670.

[10] V. Lacronique, A. Boureux, V.D. Valle, H. Poirel, C.T. Quang, M. Mauchauffe, C. Berthou, M. Lessard, R. Berger, J. Ghysdael, O.A. Bernard, A TEL-JAK2 fusion protein with constitutive kinase activity in human leukemia, Science 278 (1997) 1309–1312.

[11] Y. Iijima, T. Ito, T. Oikawa, M. Eguchi, M. Eguchi-Ishimae, N. Kamada, K. Kishi, S. Asano, Y. Sakaki, Y. Sato, A new ETV6/TEL partner gene, ARG (ABL-related gene or ABL2), identified in an AML-M3 cell line with a t(1;12)(q25;p13) translocation, Blood 95 (2000) 2126–2131.

[12] J.D. Rowley, The role of chromosome translocations in leukemogenesis, Semin. Hematol. 36 (1999) 59–72.

[13] D.R. Kaplan, F.D. Miller, Neurotrophin signal transduction in the nervous system, Curr. Opin. Neurobiol. 10 (2000) 381–391.

[14] C.D. Mackereth, M. Scharpf, L.N. Gentile, S.E. MacIntosh, C.M. Slupsky, L.P. McIntosh, Diversity in structure and function of the Ets family PNT domains, J. Mol. Biol. 342 (2004) 1249–1264.

[15] M. Barbacid, The Trk family of neurotrophin receptors, J. Neurobiol. 25 (1994) 1386–1403.

[16] M. Barbacid, Neurotrophic factors and their receptors, Curr. Opin. Cell Biol. 7 (1995) 148–155.

[17] M. Barbacid, Structural and functional properties of the TRK family of neurotrophin receptors, Ann. NY Acad. Sci. 766 (1995) 442–458.

[18] S. Jing, P. Tapley, M. Barbacid, Nerve growth factor mediates signal transduction through trk homodimer receptors, Neuron 9 (1992) 1067–1079.
[19] M.A. Lemmon, J. Schlessinger, Regulation of signal transduction and signal diversity by receptor oligomerization, Trends Biochem. Sci 19 (1994) 459–463.
[20] D.H. Wai, S.R. Knezevich, T. Lucas, B. Jansen, R.J. Kay, P.H. Sorensen, The ETV6–NTRK3 gene fusion encodes a chimeric protein tyrosine kinase that transforms NIH3T3 cells, Oncogene 19 (2000) 906–915.
[21] Q. Liu, J. Schwaller, J. Kutok, D. Cain, J.C. Aster, I.R. Williams, D.G. Gilliland, Signal transduction and transforming properties of the TEL-TRKC fusions associated with t(12;15)(p13;q25) in congenital fibrosarcoma and acute myelogenous leukemia, EMBO J. 19 (2000) 1827–1838.
[22] C. Fisher, Fibromatosis and fibrosarcoma in infancy and childhood, Eur. J. Cancer 32A (1996) 2094–2100.
[23] D.P. O'Malley, G.W. Mierau, J.B. Beckwith, D.A. Weeks, Ultrastructure of cellular congenital mesoblastic nephroma, Ultrastruct. Pathol. 20 (1996) 417–427.
[24] H.A. Oberman, Secretory carcinoma of the breast in adults, Am. J. Surg. Pathol. 4 (1980) 465–470.
[25] M. Setoyama, A. Tojo, F. Nagamura, S. Asano, M. Ishimae, M. Eguchi, N. Kamada, A unique translocation of the TEL gene in a case of acute myelogenous leukemia with inv(12)(p13q15), Blood 92 (1998) 1454–1455.
[26] J.M. Kralik, W. Kranewitter, H. Boesmueller, R. Marschon, G. Tschurtschenthaler, H. Rumpold, K. Wiesinger, M. Erdel, A.L. Petzer, G. Webersinke, Characterization of a newly identified ETV6–NTRK3 fusion transcript in acute myeloid leukemia, Diagn. Pathol. 6 (2011) 19.
[27] D. Fabbro, S. Ruetz, S. Bodis, M. Pruschy, K. Csermak, A. Man, P. Campochiaro, J. Wood, T. O'Reilly, T. Meyer, PKC412–a protein kinase inhibitor with a broad therapeutic potential, Anti-cancer Drug Des. 15 (2000) 17–28.
[28] E. Weisberg, C. Boulton, L.M. Kelly, P. Manley, D. Fabbro, T. Meyer, D.G. Gilliland, J.D. Griffin, Inhibition of mutant FLT3 receptors in leukemia cells by the small molecule tyrosine kinase inhibitor PKC412, Cancer Cell 1 (2002) 433–443.
[29] T. Fischer, R.M. Stone, D.J. Deangelo, I. Galinsky, E. Estey, C. Lanza, E. Fox, G. Ehninger, E.J. Feldman, G.J. Schiller, V.M. Klimek, S.D. Nimer, D.G. Gilliland, C. Dutreix, A. Huntsman-Labed, J. Virkus, F.J. Giles, Phase IIB trial of oral Midostaurin (PKC412), the FMS-like tyrosine kinase 3 receptor (FLT3) and multi-targeted kinase inhibitor, in patients with acute myeloid leukemia and high-risk myelodysplastic syndrome with either wild-type or mutated FLT3, J. Clin. Oncol. 28 (2010) 4339–4345.
[30] M. Eguchi, M. Eguchi-Ishimae, A. Green, T. Enver, M. Greaves, Directing oncogenic fusion genes into stem cells via an SCL enhancer, Proc. Natl. Acad. Sci. USA 102 (2005) 1133–1138.
[31] H.T. Chi, H.A. Vu, R. Iwasaki, F. Nagamura, A. Tojo, T. Watanabe, Y. Sato, Detection of exon 12 type A mutation of NPM1 gene in IMS-M2 cell line, Leuk Res. 34 (2009) 261–262.
[32] T.L. Gu, L. Popova, C. Reeves, J. Nardone, J. Macneill, J. Rush, S.D. Nimer, R.D. Polakiewicz, Phosphoproteomic analysis identifies the M0-91 cell line as a cellular model for the study of TEL-TRKC fusion-associated leukemia, Leukemia 21 (2007) 563–566.
[33] H.T. Chi, H.A. Vu, R. Iwasaki, B. Thao le, Y. Hara, T. Taguchi, T. Watanabe, Y. Sato, Green tea (−)-epigalocatechin-3-gallate inhibits KIT activity and causes caspase-dependent cell death in gastrointestinal stromal tumor including imatinib-resistant cells., Cancer Biol. Ther. 8 (2009) 1934–1939.
[34] Y. Iijima, K. Okuda, A. Tojo, N.K. Tri, M. Setoyama, Y. Sakaki, S. Asano, K. Tokunaga, G.D. Kruh, Y. Sato, Transformation of Ba/F3 cells and Rat-1 cells by ETV6/ARG, Oncogene 21 (2002) 4374–4383.
[35] G.Q. Daley, D. Baltimore, Transformation of an interleukin 3-dependent hematopoietic cell line by the chronic myelogenous leukemia-specific P210bcr/abl protein, Proc. Natl. Acad. Sci. USA 85 (1988) 9312–9316.
[36] M. Yamagishi, K. Nakano, A. Miyake, T. Yamochi, Y. Kagami, A. Tsutsumi, Y. Matsuda, A. Sato-Otsubo, S. Muto, A. Utsunomiya, K. Yamaguchi, K. Uchimaru, S. Ogawa, T. Watanabe, Polycomb-mediated loss of miR-31 activates NIK-dependent NF-kappaB pathway in adult T cell leukemia and other cancers, Cancer Cell 21 (2012) 121–135.
[37] C. Tognon, M. Garnett, E. Kenward, R. Kay, K. Morrison, P.H. Sorensen, The chimeric protein tyrosine kinase ETV6–NTRK3 requires both Ras-Erk1/2 and PI3-kinase-Akt signaling for fibroblast transformation, Cancer Res. 61 (2001) 8909–8916.
[38] J.F. Apperley, M. Gardembas, J.V. Melo, R. Russell-Jones, B.J. Bain, E.J. Baxter, A. Chase, J.M. Chessells, M. Colombat, C.E. Dearden, S. Dimitrijevic, F.X. Mahon, D. Marin, Z. Nikolova, E. Olavarria, S. Silberman, B. Schultheis, N.C. Cross, J.M. Goldman, Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta, N. Engl. J. Med. 347 (2002) 481–487.
[39] 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. Druker, 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.
[40] D.J. Propper, A.C. McDonald, A. Man, P. Thavasu, F. Balkwill, J.P. Braybrooke, F. Caponigro, P. Graf, C. Dutreix, R. Blackie, S.B. Kaye, T.S. Ganesan, D.C. Talbot, A.L. Harris, C. Twelves, Phase I and pharmacokinetic study of PKC412, an inhibitor of protein kinase C, J. Clin. Oncol. 19 (2001) 1485–1492.