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Antitumor activities of the targeted multi-tyrosine kinase inhibitor lenvatinib (E7080) against RET gene fusion-driven tumor models

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ABSTRACT

RET gene fusions are recurrent oncogenes identified in thyroid and lung carcinomas. Lenvatinib is a multi-tyrosine kinase inhibitor currently under evaluation in several clinical trials. Here we evaluated lenvatinib in RET gene fusion-driven preclinical models. In cellular assays, lenvatinib inhibited autophosphorylation of KIF5B-RET, CCDC6-RET, and NcoA4-RET. Lenvatinib suppressed the growth of CCDC6-RET human thyroid and lung cancer cell lines, and as well, suppressed anchorage-independent growth and tumorigenicity of RET gene fusion-transformed NIH3T3 cells. These results demonstrate that lenvatinib can exert antitumor activity against RET gene fusion-driven tumor models by inhibiting oncogenic RET gene fusion signaling.

1. Introduction

RET (rearranged during transfection) is a transmembrane tyrosine kinase that functions as the receptor for growth factors of the glial-derived neurotrophic factor family [1]. In papillary thyroid carcinoma, abnormal chromosomal rearrangements cause the in-frame fusion of the tyrosine kinase domain of the RET gene with heterologous genes, and the resulting RET gene fusions can lead to the expression of oncogenic driver fusion kinases [2]. Many different genes have been found to be rearranged with RET in papillary thyroid carcinoma. Coiled-coil domain containing 6 (CCDC6)-RET and nuclear receptor co-activator gene 4 (NcoA4)-RET are the most frequent variants and account for more than 90% of all RET gene fusions in papillary thyroid carcinoma.

Recently, RET gene fusions were identified in other types of cancer, including lung adenocarcinoma [3–6] and chronic myelomonocytic leukemia (CMML) [7]. In lung adenocarcinoma specifically, the kinesin family member 5B (KIF5B)-RET and CCDC6-RET fusion genes have been identified [3]. Results from preclinical studies, and the mutual exclusivity of these RET gene fusions to abnormalities in epidermal growth factor receptor (EGFR), KRAS and anaplastic lymphoma kinase (ALK), suggest that they are novel driver oncogenes in lung adenocarcinomas.

Lenvatinib is an oral multi-tyrosine kinase inhibitor that targets vascular endothelial growth factor receptors (VEGFR) 1-3, fibroblast growth factor receptors (FGFR) 1-3, RET, mast/stem cell growth factor receptor kit (SCFR), and platelet-derived growth factor receptor (PDGFR) beta. Lenvatinib has demonstrated antitumor activity with an acceptable toxicity profile in multiple phase I [8,9] and II studies [10–12]. Lenvatinib is currently being evaluated in various solid tumors, including in a phase III clinical trial in patients with radioiodine refractory differentiated thyroid cancers. Evidence from cell-free kinase assays suggests that lenvatinib inhibits RET kinase and has significant antitumor activity in RET kinase-expressing cell lines [13].

In this preclinical study, the antitumor activities of lenvatinib against RET gene fusion-driven tumor models were evaluated to assess the therapeutic potential of lenvatinib in cancers involving RET gene fusions.

2. Materials and methods

2.1. Cell lines and compounds

Nthy-ori 3-1, immortalized human thyroid epithelial cells, and A549, human lung cancer cells, were purchased from DS Pharma Biomedical Co., Ltd. (Osaka, Japan) and NCI-H1650, human lung cancer cells, were purchased from the American Type Culture Collection. These cells were cultured in RPMI1640 supplemented with

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10% fetal bovine serum (FBS). TPC-1, human papillary thyroid carcinoma cell lines, were kindly gifted from Dr. Sato in Kanazawa University and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% FBS. LC-2/ad, human lung cancer cell lines were purchased from the Riken Cell Bank (Ibaraki, Japan) and cultured in HamF12:RPMI1640 (1:1) supplemented with 15% FBS and 25 mM HEPES. NIH3T3, mouse fibroblasts, were purchased from the Riken Cell Bank and cultured in DMEM supplemented with 10% new born calf serum.

Lenvatinib was synthesized at Eisai Co., Ltd. (Tokyo, Japan) as its mesylate salt. For the cellular assays, lenvatinib was prepared in dimethyl sulfoxide (DMSO) and then diluted in the culture medium. The final concentration of DMSO in any incubation mixture did not exceed 0.2% (v/v). For the animal studies, Lenvatinib was diluted in distilled water.

2.2. Plasmid constructs and transfection

Human full-length KIF5B-RET, CCDC6-RET, NcoA4-RET and Rous sarcoma virus v-src genes (GenScript Corp., Piscataway, NJ) were chemically synthesized. These genes were amplified by polymerase chain reaction (PCR) using the primer set containing attB recombination sequences. ENTRY vectors for the Gateway cloning system (Life Technologies, Carlsbad, CA) was generated via BP Clonase reaction using the PCR products and plasmid pDONR221. Expression vectors (pCLxIP KIF5B-RET, pCLxIP CCDC6-RET, pCLxIP NcoA4-RET and pCLxIP v-src) were generated via LR Clonase reaction between each resulting ENTRY vectors and a destination vector pCLxIP-DEST. NIH3T3 cells were transfected with these plasmids using Lipofectamine 2000 (Life Technologies). Stable transfectants were selected with 1 μg/ml puromycin.

2.3. Western blotting

Nthy-ori 3-1 cells were transfected either with KIF5B-RET, CCDC6-RET or NcoA4-RET expressing plasmids using X-tremeGENE9 (Roche Diagnostics K. K., Tokyo, Japan). Next day, cells were treated with lenvatinib for 1 h and lysed with lysis buffer containing 4% sodium dodecyl sulfate, 30% glycerol, 125 mM Tris–HCl (pH 6.8), 10% β-mercaptoethanol, 1 mM phenylmethanesulfonylfluoride, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4 and analyzed by Western blotting. TPC-1 cells and LC-2/ad cells were plated in 10 cm dishes. After overnight incubation, the cells were treated with lenvatinib for 3–4 h and lysed and analyzed by Western blotting. Western blotting was performed using standard procedures with HYBOND-ECL nitrocellulose membrane (GE Healthcare, Buckinghamshire, UK) and Immobilon Western chemiluminescent HRP Substrate (Millipore Corporation, Massachusetts, USA). Antibodies to RET, phospho-RET (Y905), ERK1/2, phospho-ERK1/2 (T202/Y204), AKT and phospho-AKT (S473) were purchased from Cell Signaling Technology (Massachusetts, USA). Antibody to beta-actin was purchased from Sigma–Aldrich (Missouri, USA).

2.4. Cell growth assay

Cells ($n = 3$ assays) were plated at 300–500 cells/well in a 96-well plate. After overnight incubation, the cells were treated with serial dilutions of lenvatinib for 7 days. Viable cell titer relative to untreated cells was determined with Cell Counting Kit-8 (Dojindo Laboratories, Japan) according to the manufacturer's protocol.

2.5. Soft agar colony formation assay

Surface of 24-well plates ($n = 4$ assays) was covered with 0.3 ml of 0.66% Noble agar (Difco Laboratories Inc., Detroit, MI, USA) in tissue culture medium (agar/medium). Transformed NIH3T3 cells in the tissue culture medium (2×10^4 cells/ml) was mixed with equal volume of agar/medium and seeded onto the bottom agar layer at 0.3 ml/well. Agar/medium was overlaid at 0.3 ml/well as an upper agar layer. The upper agar was covered with 0.6 ml of culture medium containing test compounds. After 10–21 days incubation, 0.1 ml of 3.3 mg/ml 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide in PBS was added and incubated for 5 h to stain viable cells. The areas of the stained colonies were measured with an image analyzer program (Spectrum, Mitani Corp., Fukui, Japan).

2.6. Antitumor studies

Transformed NIH3T3 cells were injected subcutaneously into the flank of female BALB/c nu/nu athymic mice. Tumor volumes were determined as length × (diameter)^2/2, where length was the longest dimension and diameter was the shortest dimension. When tumor volume reached 100–200 mm^3, mice were randomized into groups with approximately equal mean tumor volumes. For antitumor studies ($n = 6$), mice received 10 ml/kg oral administration of lenvatinib or vehicle once daily for 10 days. On the day after the last treatment, tumors were collected and fixed in 10% buffered formalin and paraffin-embedded for microvessel count. For pharmacodynamics analysis ($n = 5$), mice were treated with lenvatinib similarly as for the anti-tumor studies. Two hours later, tumors were excised and tumor lysates were analyzed by Western blotting. All experiments were approved by the Animal Care and Use Committee at the Eisai Tsukuba Research Laboratories (Ibaraki, Japan).

2.7. Microvessel count

Tumor tissue sections from paraffin-embedded block were stained with a rat anti-mouse CD31 monoclonal antibody (BD Biosciences, San Jose, CA, USA) and counter-stained with haematoxylin. Five regions with highest densities of microvessels in each tumor tissue sections were selected. Microvessel density was determined as the mean value of numbers of microvessel in the selected regions.

2.8. Statistical analysis

Data are expressed as mean ± standard deviation. The differences in colony formation, tumor volume, and microvessel density were analyzed by the Dunnett multiple comparison test. A value of $p < 0.05$ (2 sided) was considered statistically significant. Statistical analyses were performed using GraphPad Prism version 5.04 (GraphPad Software, Inc., La Jolla, CA, USA).

3. Results

3.1. Lenvatinib inhibits RET gene fusion kinases in intact cells

We have previously reported that lenvatinib inhibits RET kinase in cell free assay with Ki value of 1.5 nmol/L [13]. To determine whether lenvatinib could inhibit the kinase activity of RET gene fusion kinases in intact cells, we transiently transfected Nthy-ori 3-1, immortalized human thyroid epithelial cells with plasmids encoding either KIF5B-RET, CCDC6-RET or NcoA4-RET. After treatment with lenvatinib, cells were lysed and phosphorylation status of tyrosine 905 (Y905) of exogenously expressed RET gene fusion kinases was determined by Western blot analysis. Y905 is located in the activation loop of the RET kinase domain and reported to be auto-phosphorylated by RET gene fusion kinases in the absence of ligand [5,14]. As shown in Fig. 1, Y905 of all 3 RET gene fusion kinases were phosphorylated in Nthy-ori 3-1 cells. Lenvatinib treatment decreased the phosphorylation of Y905 of these RET gene fusion kinases in a dose range that was nearly similar. These results indicate that these RET gene fusion kinases were similarly sensitive to lenvatinib.

3.2. Inhibition of oncogenic CCDC6-RET signaling in a human cancer cell lines

We next investigated the effects of lenvatinib on human cancer cell lines harboring RET gene fusions. The papillary thyroid

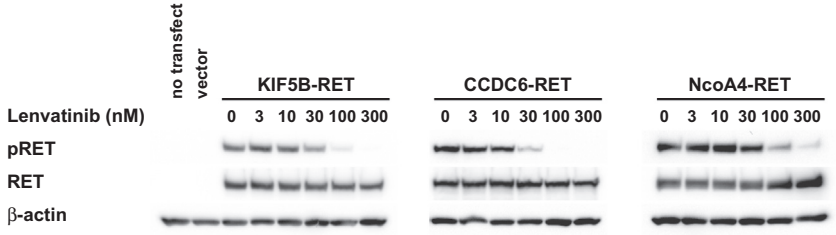

Fig. 1. Inhibition of RET gene fusion proteins by lenvatinib in intact cells. Nthy-ori 3-1 cells were transfected with either KIF5B-RET, CCDC6-RET or NcoA4-RET expressing plasmids and treated with lenvatinib for 1 h. Cells were lysed and analyzed by Western blotting for indicated markers.

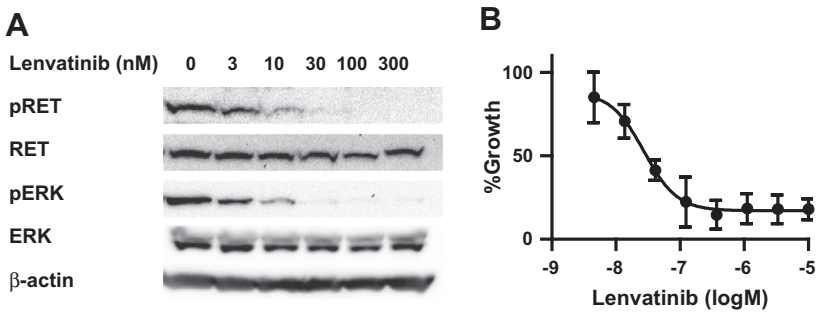

Fig. 2. Lenvatinib inhibits oncogenic CCDC6-RET signaling in thyroid cancer cell line, TPC-1. (A) TPC-1 cells were treated with lenvatinib for 3 h. Cells were lysed and analyzed by Western blotting for indicated markers. (B) Relative growth of TPC-1 cells after treatment with lenvatinib in serum-free condition. Data points represent the mean of 3 wells; bars represent the SD.

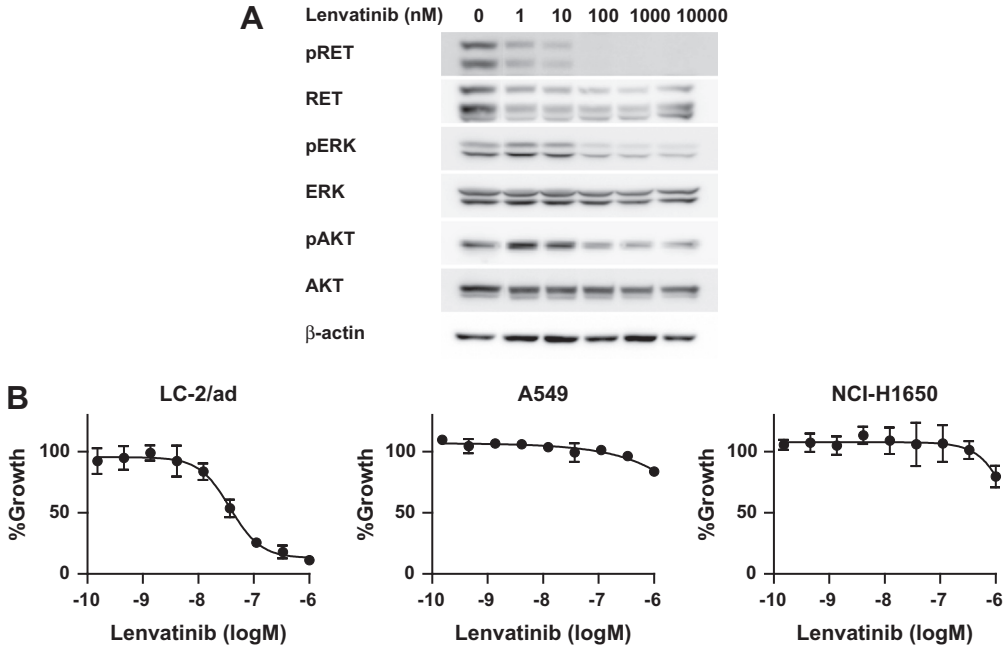

Fig. 3. Lenvatinib inhibits oncogenic CCDC6-RET signaling in lung cancer cell line, LC-2/ad. (A) LC-2/ad cells were treated with lenvatinib for 4 h. Cells were lysed and analyzed by Western blotting for indicated markers. (B) Relative growth of CCDC6-RET positive (LC-2/ad) and negative (A549 and NCI-H1650) lung cancer cell lines after treatment with lenvatinib. Data points represent the mean of 3 wells; bars represent the SD.

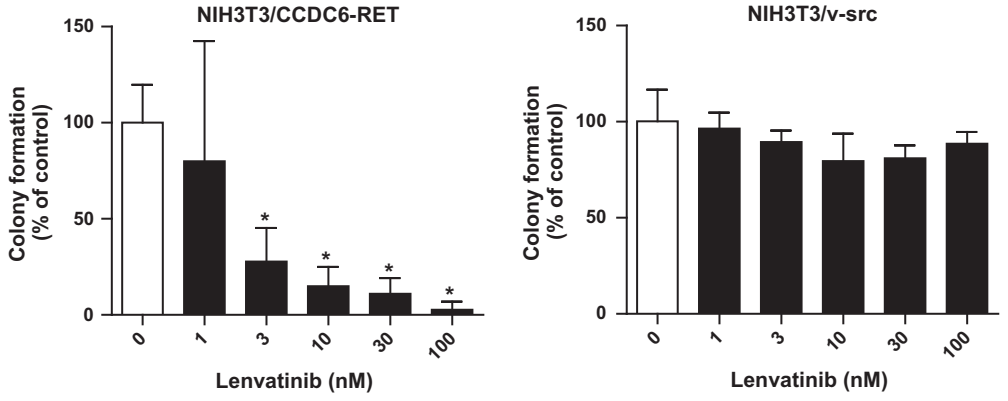

Fig. 4. Lenvatinib inhibits RET gene fusion induced anchorage-independent growth. NIH3T3 cells were transformed by either CCDC6-RET or v-src expressing plasmid to induce anchorage-independent growth. Bar graph showing the percentage (±SD) of colonies formed after treatment with the indicated amounts of lenvatinib with respect to those formed by control cells (0 nM). $^{*}p < 0.01$ compared with control cells (the Dunnett multiple comparison test).

carcinoma TPC-1 cells bear CCDC6-RET rearrangement [15]. To determine whether lenvatinib could inhibit the aberrant kinase activation of CCDC6-RET in TPC-1 cells, we treated TPC-1 cells with lenvatinib and analyzed phosphorylation status of Y905 of CCDC6-RET by Western blotting. The phosphorylation of CCDC6-RET was reduced by lenvatinib and almost completely abrogated by 30 nM lenvatinib (Fig. 2A). To determine whether lenvatinib affected signaling, activation of ERK was monitored by Western blotting with an antibody specific for p42- and p44-ERKs phosphorylated at threonine 202 and tyrosine 204. ERK phosphorylation was also reduced by lenvatinib treatment in similar dose response to reduction of RET phosphorylation (Fig. 2A), indicating that lenvatinib suppressed the oncogenic signaling of CCDC6-RET in TPC-1.

Next, we studied the effects of lenvatinib on the growth of TPC-1 cells in cell growth assays under serum-free conditions. Lenvatinib inhibited the growth of TPC-1 cells with an IC_{50} value of 27 nM (Fig. 2B).

Lung cancer LC-2/ad cells were recently reported to harbor the CCDC6-RET re-arrangement [16]. To evaluate effects of lenvatinib on the oncogenic signaling of CCDC6-RET in LC-2/ad, we treated LC-2/ad cells with lenvatinib and conducted Western analysis. Lenvatinib suppressed the phosphorylation of RET, ERK and AKT in similar dose response (Fig. 3A). Next, we studied the effects of lenvatinib on the growth of LC-2/ad *in vitro*. As shown in Fig. 3B, lenvatinib inhibited growth of LC-2/ad with an IC_{50} value of 48 nM. Conversely, lenvatinib had almost no effect on cell growth of 2 other lung adenocarcinoma cell lines, A549 and NCI-H1650. We also assessed the effects of lenvatinib on cell cycle in LC-2/ad cells. Lenvatinib significantly decreased the percentage of cells in S phase at 100 nM (Fig. S1), suggesting that inhibition of the oncogenic signaling of CCDC6-RET caused G1 phase arrest in LC-2/ad.

Because lenvatinib targets several tyrosine kinases, the growth suppression effect of lenvatinib in TPC-1 and LC-2/ad may be enhanced by inhibition of endogenous kinases. To assess this possibility, global phosphorylation of receptor tyrosine kinases (RTK) was determined by using phospho-RTK arrays. No significant activation of target kinases of lenvatinib (VEGFRs, FGFRs, SCFR and PDGFRs) was observed in both cell lines (Fig. S2).

3.3. Lenvatinib Inhibits transforming activity of RET gene fusions

RET gene fusions were reported to transform NIH3T3 fibroblasts [5,17]. To further evaluate the inhibitory activity of lenvatinib towards oncogenic activities of RET gene fusions, we generated stable transfectants of NIH3T3 cells with plasmids encoding either KIF5B-RET or CCDC6-RET (NIH3T3/KIF5B-RET and NIH3T3/CCDC6-RET). As a control, we also generated v-src-transformed NIH3T3 cells (NIH3T3/v-src). NIH3T3/CCDC6-RET and NIH3T3/v-src showed anchorage-independent growth in soft agar colony formation assay (Fig. 4). The inhibitory activity of lenvatinib on colony formation of NIH/CCDC6-RET and NIH/v-src was evaluated. Treatment with lenvatinib suppressed the anchorage-independent growth of NIH/CCDC6-RET, whereas lenvatinib had no effect on the growth of NIH/v-src (Fig. 4). NIH3T3/KIF5B-RET also formed colonies in soft agar. However, the number of colonies formed by NIH3T3/KIF5B-RET was much lower than those of NIH/CCDC6-RET and NIH/v-src. Western blot analysis revealed an exceedingly low expression level of RET gene fusion in NIH3T3/KIF5B-RET (data not shown) and this was considered to be a potential causative factor for the lower efficiency of colony formation observed.

3.4. Antitumor activities of lenvatinib in RET gene fusion driven tumor models in mice

Finally, to assess the therapeutic potential of lenvatinib, we evaluated the inhibitory activities of lenvatinib towards the tumorigenicity of the transformed NIH3T3 cells in athymic mice. After tumors were established, mice were treated with lenvatinib daily for 10 days. Lenvatinib treatment suppressed growth of tumors derived from NIH3T3/KIF5B-RET and NIH3T3/CCDC6-RET (Fig. 5). In contrast, lenvatinib had no effect on the tumorigenicity of NIH3T3/v-src. Lenvatinib treatment was well tolerated, as determined by stable body weights during the treatment period (Fig. S4). Because lenvatinib has exhibited inhibitory activity on VEGFR-2 and suppresses angiogenesis in human xenograft models [13], we evaluated the effect of lenvatinib on angiogenesis in NIH3T3/KIF5B-RET and NIH3T3/v-src xenografts (Fig. 6). Treatment with lenvatinib resulted in significant decrease in microvessel density in both NIH3T3/KIF5B-RET and NIH3T3/v-src xenografts.

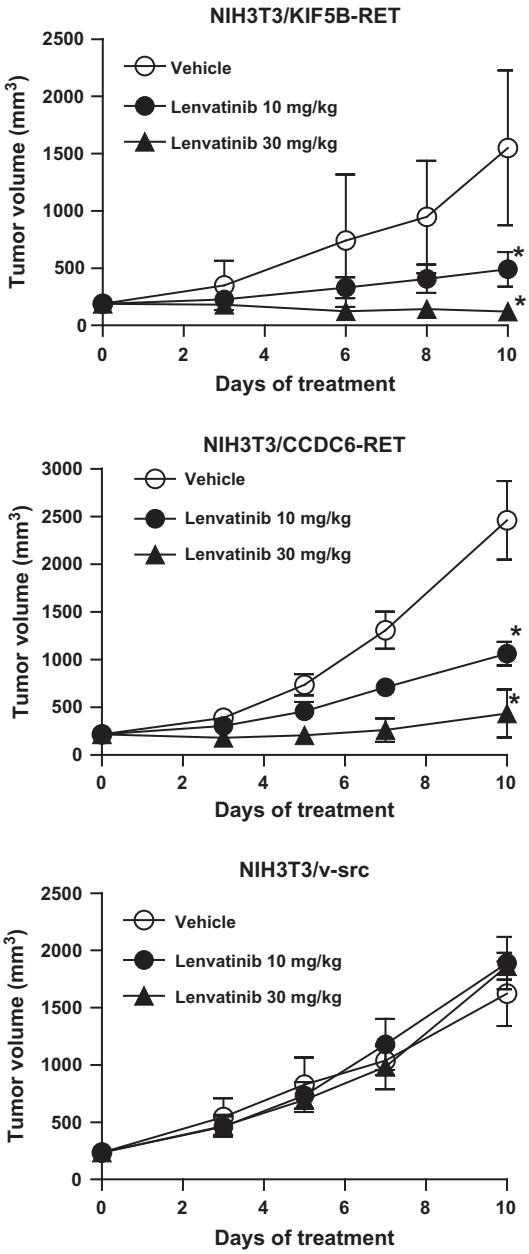

Fig. 5. Antitumor activity of lenvatinib in RET gene fusion driven models. NIH3T3 cells transformed by either KIF5B-RET, CCDC6-RET or v-src were injected s.c. into nude mice. After tumors were established, mice were orally treated with lenvatinib daily for 10 days. Data points represent mean (±SD) of 6 animals. $^{*}p < 0.05$ compared with vehicle on day 10 (the Dunnett multiple comparison test).

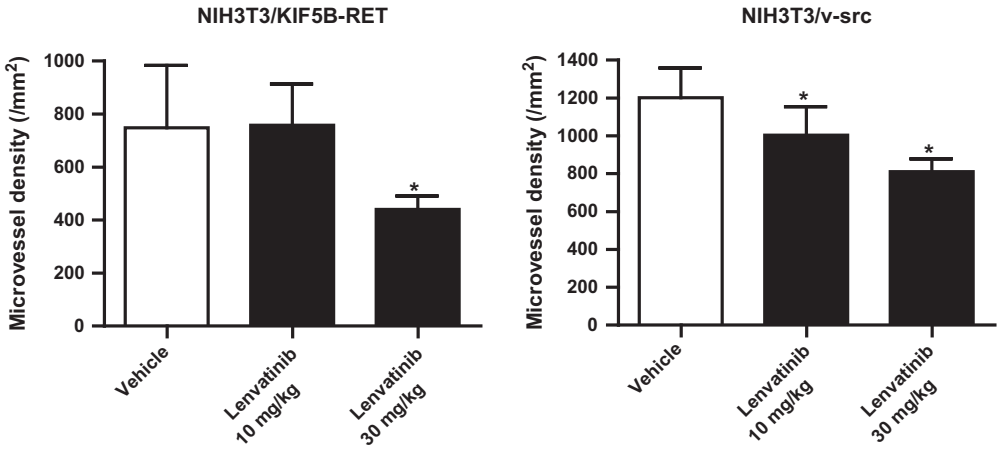

Fig. 6. Antiangiogenesis activity of lenvatinib in transformed NIH3T3 xenograft models. NIH3T3 cells transformed by either KIF5B-RET or v-src were injected s.c. into nude mice. After tumors were established, mice were orally treated with lenvatinib daily for 10 days. Data points represent mean (±SD) of 6 animals. $^{*}p < 0.05$ compared with vehicle on day 10 (the Dunnett multiple comparison test).

To determine whether lenvatinib inhibited the kinase activity of RET fusion kinases in the animal model, mice bearing NIH3T3/KIF5B-RET tumors were orally treated with lenvatinib and the status of KIF5B-RET signaling was analyzed by Western blotting. As shown in Fig. 7, phosphorylation of Y905 of KIF5B-RET was reduced by lenvatinib treatment at 10 mg/kg and 30 mg/kg. ERK phosphorylation was also reduced by lenvatinib treatment. Taken together, these results indicate that lenvatinib demonstrated antitumor activity against RET gene fusion-transformed NIH-3T3 cells with simultaneous RET inhibition.

4. Discussion

In this study lenvatinib, an oral multi-tyrosine kinase inhibitor, inhibited RET gene fusion kinases. We have also shown that lenvatinib inhibits oncogenic signaling of RET gene fusions and exerts antitumor activities in RET gene fusion-driven tumor models.

More than 13 fusion partner genes of RET have been reported to date. In the case of ALK gene fusions, partner genes may be important determinants of sensitivity to inhibitors [18]. In the present study, we have shown that 3 RET gene fusions, KIF5B-RET, CCDC6-RET and NcoA4-RET were similarly sensitive to lenvatinib in cellular assays at concentrations in the 30 to 100 nM range. Given that CCDC6-RET and NcoA4-RET account for more than 90% of RET gene fusions in papillary thyroid carcinoma [2] and RET gene fusions other than KIF5B-RET and CCDC6-RET are not reported in lung adenocarcinomas, lenvatinib may inhibit RET gene fusion kinases in these carcinomas in a similar dose range. Recently, 2 novel

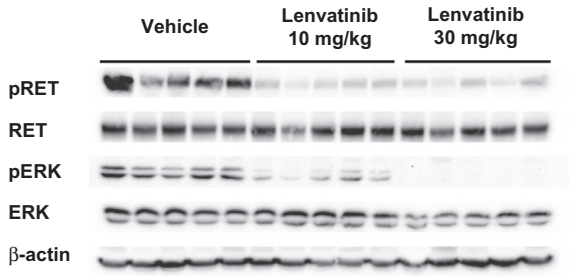

Fig. 7. Lenvatinib inhibits KIF5B-RET in xenografts in mice. Mice bearing with NIH3T3/KIF5B-RET tumors were orally treated with lenvatinib. Two hours later, tumors were excised and tumor lysates were analyzed by Western blotting for indicated markers.

RET gene fusions, BCR-RET and FGFR10P-RET, were identified in CMML [7]. Preclinical studies suggest these fusions are mutational drivers in CMML [7]. Based on the activities of lenvatinib on RET gene fusions shown in the present study, it is likely that lenvatinib would effectively target these novel RET gene fusions in CMML. However, further studies are warranted to assess the therapeutic potential of lenvatinib in CMML with RET gene fusions.

In the study of lung adenocarcinoma cell lines, we found that lenvatinib inhibited growth of CCDC6-RET positive LC-2/ad cells but not those of A549 and NCI-H1650. A549 and NCI-H1650 are likely negative for RET gene fusions. A549 and NCI-H1650 cells reportedly carry KRAS and EGFR mutations, respectively [19]. Mutual exclusivity of these oncogenes to RET gene rearrangement in lung cancer has been reported [5], therefore it is unlikely that these cells carry RET gene fusions.

Secondly, Matsubara and colleagues reported that RET gene expression levels in these cells were lower than those observed in LC-2/ad and fusion-specific RT-PCR. Amplification of PCR products in A549 cells failed to detect evidence of CCDC6-RET or KIF5B-RET [16]. Among 11 lung cancer cell lines tested, LC-2/ad growth was found to be most sensitive to lenvatinib (data not shown), most likely due to lenvatinib targeting of KIF5B-RET. Taken together, these results suggest the possibility that lenvatinib is selective in inhibiting the RET fusion proteins within adenocarcinoma cell lines. Further exploration of lenvatinib's selective effect on the RET fusion proteins in these lung cancer lines is warranted.

Pharmacokinetics (PK) of lenvatinib in patients with advanced solid tumors was reported [8]. In this study, lenvatinib was administered orally on a once-daily continuous schedule in 28-day cycles and the maximum tolerated dose (MTD) was defined as 25 mg once daily. Maximal plasma concentration, time to maximal plasma concentration and plasma half-life at MTD was 598.0 ng/ml (1401 nM), 1.5 h and 5.3 h, respectively. In the present study we have shown that lenvatinib inhibited oncogenic RET signaling in cellular assays at concentrations in the 30–100 nM range. Based on the PK data, continuous suppression of RET fusion proteins is expected by practical oral administration of lenvatinib in human.

Lenvatinib has inhibitory activity on VEGFR-2 and suppresses angiogenesis in human xenograft models [20]. Antiangiogenic activity of lenvatinib was confirmed in a phase I clinical study by using circulating endothelial cells as a biomarker [9]. The antitumor activities of lenvatinib in papillary thyroid cancer and lung adenocarcinoma cells positive for RET gene fusion kinases

demonstrated in the current study are distinct from the previously observed antiangiogenic activity of lenvatinib based on several observations. Although lenvatinib suppressed angiogenesis in NIH3T3/v-src xenografts, it had very little effect on the tumor growth of these xenografts. Additionally, lenvatinib suppressed the tumor growth of NIH3T3/KIF5B-RET and NIH3T3/CCDC6-RET xenografts. These results suggest that the observed antitumor activities of lenvatinib in RET gene fusion driven NIH3T3 models are derived predominantly from direct inhibition of transformed cell growth. Based on these observations, we plan to evaluate potential synergy between lenvatinib antiangiogenic activity and RET gene fusion inhibition in xenograft models derived from RET gene fusion positive human cancer cell lines.

Kinase gene fusions represent an important class of oncogenes associated with solid tumors and leukemia. The rationale for targeting kinase gene fusions by small molecular inhibitors was first supported by the significant hematologic and cytogenetic responses to imatinib in BCR-ABL-positive chronic myeloid leukemia patients [21]. Recently, crizotinib demonstrated clinical benefit in ALK gene fusion positive non-small cell lung cancer (NSCLC) patients, leading to its approval in 2011 by the Food and Drug Administration [22]. The clinical success of the ALK and BCR-ABL inhibitors (i.e., crizotinib) suggests that targeting specific fusion proteins is a promising therapeutic strategy for treatment of multiple cancers. To the best of our knowledge, 3 kinase genes, ALK, RET, and ROS1 [23], have been associated with oncogenic gene fusions in NSCLC. Data presented in this report suggest that the antitumor activities of lenvatinib against RET gene fusion-transformed cells are conferred by RET inhibition. Recently, promising clinical effects of RET targeting therapy using cabozantinib [24] and vandetanib [25] were reported. A study using a Drosophila screening system suggested that the antitumor activity and toxicity of RET inhibitors were modified by the "off-target" kinase inhibition profiles [26]. Because, lenvatinib, cabozantinib and vandetanib have different kinase inhibitory profiles [27], further investigation to elucidate how these differences in kinase inhibitory profiles affect the antitumor activity and toxicity is anticipated. Taken together, these findings warrant further evaluation of lenvatinib in patients with tumors harboring RET gene fusions, such as KIF5B-RET- and CCDC6-RET-positive lung adenocarcinomas. A clinical trial of lenvatinib in this patient population is currently being planned.

Conflict of interest

All authors are employee of Eisai Co., Ltd.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.canlet.2013.07.007.

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