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Extended Abstract

Pharmaceutical Sciences 2018: Lenvatinib exhibits antineoplastic activity in anaplastic thyroid cancer in vitro and in vivo-Silvia Martina Ferrari-University of Pisa

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Abstract

Introduction: Lenvatinib is an oral, multi-targeted tyrosine kinase inhibitor of the vascular endothelial growth factor receptors 1–3, fibroblast growth factor receptors 1–4, PDGFRα, RET and v-kit Hardy-Zuckerman feline sarcoma viral oncogene homolog signalling networks involved in tumour angiogenesis.

In vitro studies have been evaluated lenvatinib in preclinical models. Lenvatinib decreased the auto-phosphorylation of KIF5B-RET, CCDC6-RET and NcoA4-RET, by inhibited the proliferation of CCDC6-RET human thyroid and lung cancer cell lines and blocked the tumorigenicity of RET gene fusion-transformed NIH3T3 cells. *In vivo* phase II and phase III studies patients with aggressive DTC not responsive to radio-iodine have demonstrated that the administration of lenvatinib is associated with an improvement in progression-free survival compared with placebo following the results of this phase III study, lenvatinib has been approved for the treatment of patients with locally recurrent or metastatic, progressive, radioactive iodine refractory DTC. In the present study, we aimed to evaluate the anti-neoplastic activity of lenvatinib in ATC continuous cell lines and in primary ATC cell cultures both *in vitro* and *in vivo*.

Materials & Methods

Chemicals and supplements

Lenvatinib was evaluated in primary ATC cell cultures, in 8305C cells and AF cells, and in AF cells in CD nu/nu mice. Chemicals and supplements were obtained from Sigma-Aldrich. RPMI-1640 medium was purchased from Gibco. PCR reagents for quantitative PCR were purchased from the Applied Biosystems.

Thyroid tissues

Thyroid samples were surgically collected from the 9 ATC patients and from 5 healthy subjects under-going the parathyroidectomy. The diagnosis was made on the basis of clinical and histological criteria by a recognized laboratory. By immunohistochemistry it was demonstrated that TSH receptor, sodium symporter, thyroperoxidase and thyroglobulin were not expressed in thyroid tissues.

Cell cultures

- Human primary ATC cell cultures
- Thyroid follicular cell culture
- AF cell line
- Evaluation of cell viability and proliferation
- Apoptosis: Hoechst uptake and Annexin V binding assay
- Migration and invasion tests

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ELISA tests in ATC cells

- Phospho-EGFR inhibition cell-based assay
- ERK1/2 and Akt ELISA
- Cyclin D1 protein expression is quantified in lenvatinib-treated ATC cells

In vivo studies

Animals and treatment

Six-week-old CD nu/nu male mice, provided by Envigo, were housed in microisolator cages on vented racks and manipulated using aseptic techniques. Housing and procedures involving animals were conducted according to the protocol approved by the Academic Organization Responsible for Animal Welfare at the University of Pisa, according to the Italian law D.lgs. 26/2014, and with the approval of the Italian ministry of Health.

Tumour tissue: Immunohistochemistry and micro vessel density determination

Neoplastic samples from the two treatment groups were weighed, then fixed in formalin and subsequently embedded into paraffin. Sections of 5-μm thickness were stained by haematoxylin and eosin, as previously described.

Statistical analysis

Experiments were conducted in triplicate from each subject and mean of the samples were reported for TFC and ATC cells. One-way ANOVA, Mann-Whitney U is test were used to compare mean group values for normally distributed variables. The χ^2 test was used to compare the group proportions. Post hoc comparisons on normally distributed variables were performed using the Bonferroni-Dunn test.

Results

In vitro studies in ATC cells

Evaluation of cell proliferation

Data was obtained from the WST-1 assay in TFC cell's following lenvatinib treatment demonstrated a slight but significant reduction in the proliferation rate vs control group both at 1 h with lenvatinib 10 μM, 25 μM and 50 μM and at 2 h with lenvatinib 10 μM, 25 μM and 50 μM. Cell counting confirmed these results: after 1 h, the cell number was 10,150±620/100 μl/well in the TFC control; 9,642±1,100 (95%) with lenvatinib 10 μM; 9,238±960 (91%) with lenvatinib 25 μM; and 8,625±950 (85%) with lenvatinib 50 μM; after 2 h, the cell number was 17,500±820/100 μl/well; 15,925±1,120 (91%) with lenvatinib 10 μM; 14,874±1,060 (85%) with lenvatinib 25 μM; and 14,350±980 (82%) with the lenvatinib 50 μM.

Proliferation and BRAF

Proliferation was inhibited in a similar manner in ATC from tumours in the presence/absence of ^{V600E}*BRAF* mutation.

Apoptosis evaluation

Lenvatinib is a dose-dependent increased apoptotic ATC cells. The Annexin V assay corroborated these results.

Migration and invasion tests

After reaching subconfluence, primary ATC cell cultures were treated with the increasing concentrations of lenvatinib. Lenvatinib inhibited migration and invasion, as evaluated by the Transwell chamber.

Inhibition of EGFR

Lenvatinib significantly and dose-dependently decreased by the phosphorylated form of EGFR in ATC cell lysates.

Inhibition of Akt or ERK1/2 phosphorylation

Phosphorylated/non-phosphorylated Akt or ERK1/2 proteins in lenvatinib-treated samples were significantly reduced in the ATC cell cultures.

Lenvatinib reduces cyclin D1 protein levels

Lenvatinib reduced cyclin D1 concentrations compared with vehicle-treated cells.

In vitro studies in 8305C and AF cells

Lenvatinib had a dose-dependent antiproliferative activity in 8305C cell and in AF cells. Following exposure to lenvatinib 10 µM, 19.8% of cells were apoptotic and with lenvatinib 25 or 50 µM, 25 and 30.8% of cells were apoptotic, respectively.

In vivo studies

Lenvatinib reduces AF tumor growth with no weight loss

Lenvatinib significantly reduced tumor growth, from day 7 after treatment started, compared with the controls. Notably, no loss of weight was observed throughout the course of the experiment indicating that lenvatinib treatment was well tolerated.

Discussion:

Research on the effects of TKIs for the treatment of ATC is ongoing. In the present study, we demonstrated that lenvatinib inhibited primary ATC cell cultures proliferation *in vitro*, while also increasing apoptosis and inhibiting migration and invasion. In addition, lenvatinib inhibited the proliferation of 8305C and AF cells *in vitro*, while also increasing apoptosis and reduced AF cell tumor growth in CD nu/nu mice with no toxicity. These results were consistent with previous studies that identified an ability of lenvatinib to inhibit tumor growth of ATC cell lines *in vivo* and to disrupt angiogenesis by decreasing vascular permeability.

In the present study, we revealed for the first time the antitumoral effect of lenvatinib, a multi-targeted kinase inhibitor, in primary human ATC cell cultures obtained from patients. These findings could open the way to the clinical use of lenvatinib in the treatment of patients with ATC.

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