NEXTFRONTIERS TOCURECANCER2022
DE 22 A 25/06 - 100% ONLINE

A.C.Camargo Cancer Center
Especializado em Vida

NT157 potentiates the reduction of cell viability induced by gefitinib treatment in lung cancer cell models

Lívia Bassani Lins de Miranda (1), Keli Lima (1), Juan Luiz Coelho-Silva (2), Fabiola Traina (2), Susumu S. Kobayashi (3), João Agostinho Machado-Neto (1)

(1) Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil;
(2) Department of Medical Images, Hematology and Clinical Oncology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil;
(3) Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, 330 Brookline Avenue, E/CLS-407, Boston, MA 02215, USA

Introduction

Lung cancer is one of the most common types of tumors in the world, and has the highest mortality. Studies show that the over activation of IGF1R and AXL has been associated with resistance to EGFR inhibitors, commonly used drugs against lung cancer. NT157 is a small synthetic molecule described as a pharmacological inhibitor of IGF1R and STAT3. Studies on different cancer models reported that the mechanism of action involves the activation of ERK which phosphorylates IRS1/2, leading to its degradation. It had also been described that NT157 modulates the expression of AXL. There are no studies on the effects of NT157 in lung cancer models, furthermore, there are no studies regarding NT157-based combination therapy.
The current study aims to analyze the effects of NT157 in lung cancer cells and further increase the knowledge about the drug as an antineoplastic agent.

Materials and Methods

For the study the lung cancer cell lines H1299 and H460 were utilized. For the protein expression analysis, cells were treated with NT157 at the concentrations of 6.4 and 12.5 µM or vehicle, and a Western blot assay was performed. The protein expression was also analyzed in a combination therapy with the JNK inhibitor SP600125 at the concentration of 20 µM.
A qPCR assay was performed to assess gene expression upon NT157 treatments at the concentrations of 6.4 and 12.5 µM or vehicle.
The synergy analyzes was performed by SRB assay, a cell viability assay, in which cells were treated with NT157 in the concentrations of 0.4, 0.8, 1.6, 3.2 and 6.4 µM combined with the EGFR inhibitor Gefitinib at the concentrations of 3.2, 6.4, 12, 25 and 50 µM.

Results

In lung cancer cell models, treatments with NT157 reduced expression of IRS1 and AXL and inhibited the phosphorylation of p38 MAPK, AKT, and 4EBP1, which are related to cell growth, survival and proliferation. Treatments also induced the expression of γH2AX and the cleavage of PARP1, indicating that the cells were undergoing apoptosis induced by NT157. In H1299 cells, treatments enhanced the expression of IRS2 and phosphorilated JNK and ERK, in the other hand, in H460 cells NT157 caused a reduction in the expression of IRS2.

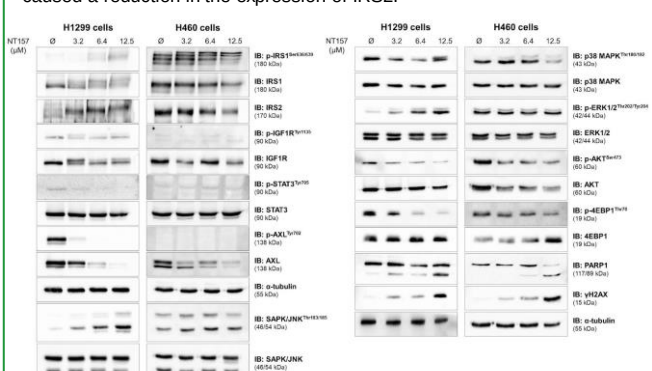

Image 1: Western blot analysis for p-IRS1$^{Ser636/639}$, IRS1, IRS2, p-IGF1R^{Tyr1136}, IGF1R, p-STAT3^{Tyr705}, STAT3, p-AXL^{Tyr702}, AXL, p-SAPK/JNK$^{Thr183/185}$, SAPK/JNK, p-p38 MAPK$^{Thr180/182}$, p38 MAPK, p-ERK1/2$^{Thr202/Tyr204}$, ERK1/2, p-AKT^{Ser473}, AKT, p-4EBP1^{Thr70}, 4EBP1, PARP1, and γH2AX in total cell extracts from H1299 and H460 cells treated with vehicle or NT157 (3.2, 6.4, and 12.5 µM) for 24 hours.

Previous studies indicated that IRS1 and IRS2 phosphorylation triggered by NT157 is induced by ERK, which was not observed in lung cancer cell models, suggesting that other MAPK may be involved in the process. The protein JNK, similarly to ERK, also phosphorylate IRS1 and IRS2, indicating it could be related to the process. To verify the hypothesis, the compound SP600125, a selective JNK inhibitor, was used alone or in combination with NT157. NT157 induced IRS1 and IRS2 phosphorylation, activated JNK, and increased the expression of c-JUN, while the treatments using SP600125 prevented NT157-induced IRS1 and IRS2 phosphorylation in both lung cancer cells studied. Reduced c-JUN phosphorylation confirmed the efficiency of SP600125. Our data indicated that JNK has a key role in the NT157-induced IRS1 and IRS2 phosphorylation, revealing a novel axis involved in the mechanism of action of the drug.

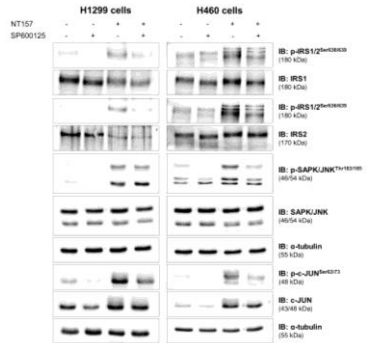

Image 2: Western blot analysis of indicated protein involved in IRS1/2-JNK axis for H1299 and H460 cells treated with vehicle, SP600125 and/or NT157 for 6 h. NT157 induces IRS1/2 phosphorylation, which is prevented by SP600125. Expression of p-c-JUN$^{Ser63/73}$ was used as a control for SP600125 efficacy.

NT157 treatments decreased the expression of oncogenes *BCL2, CCND1, MYB*, and *MYC* and increased genes related to cellular stress and apoptosis, *JUN, BBC3, CDKN1A, CDKN1B, FOS*, and *EGR1*. H1299 cells had an increase of *NFKB1* and *PTEN* expression, which was the opposite effect that happened to H460 cells. The results indicated that NT157 favors a tumor-suppressive gene expression in both cell lines.

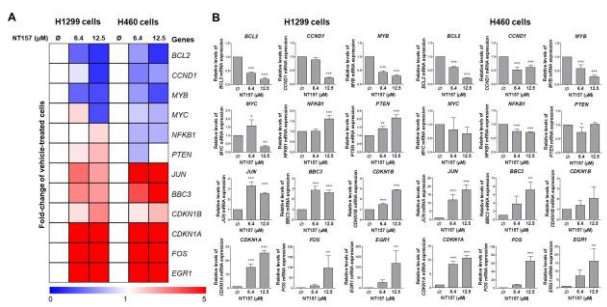

Image 3: (A) Heatmap of the gene expression of H1299 and H460 cell lines treated with NT157 or vehicle. (B) The bar graphs represent the mean ± SD of at least three independent experiments. *p < 0.05, **p < 0.1 and ***p < 0.001, ANOVA and Bonferroni post-test.

The overactivation of IGF1R and AXL have been associated with resistance to EGFR inhibitors, which are first line treatments for lung cancer. Combination treatments utilizing NT157 and Gefitinib were performed to assess if the effect of both drugs together would be any different when compared to monotherapies. Of note, NT157 in combination with Gefitinib presented potentiating effects in the reduction of cell viability in both cell lines.

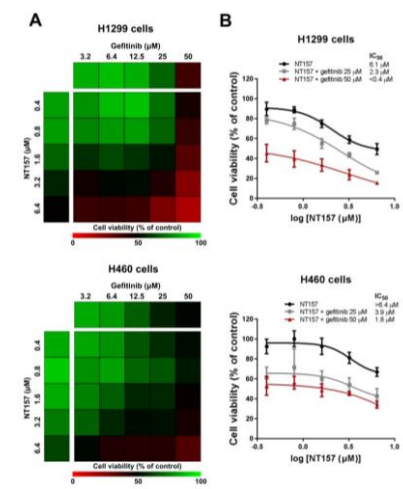

Image 4: (A) The heatmap indicates the cell viability of H1299 and H460 cells treated with graded concentrations of NT157 and Gefitinib alone or in combination with each other for 48 h **(B)** Dose-response cytotoxicity for NT157 in combination with Gefitinib (25 or 50 µM) for H1299 and H460 cells. Values are expressed as the percentage of viable cells for each condition relative to untreated controls.

Conclusion

Our data indicated that in lung cancer cell models, NT157 decreases the expression of proteins related to cell proliferation and survival and enhances the expression of proteins related to apoptosis. NT157 also favors a tumor-suppressive gene expression network .Our findings also suggest a novel mechanism of action for NT157, which involves the IRS1 and IRS2 phosphorylation by JNK. Lastly, the current study shows that the combination of NT157 and Gefitinib potentiates the antineoplastic effects compared to monotherapy in lung cancer.

Contact

Presenting author: liviamirands@usp.br; (11) 98422-9860; Av. Professor Lineu Prestes, 1524, Lab 210

Supported by: CNPq CAPES FAPESP