INTRODUCTION

Lung carcinoid (LC) tumors are quite rare (only 1-2% of all lung cancers) and classified in typical carcinoids (TC) and atypical carcinoids (AC). Although these tumors usually grow slowly, LCs develop distant metastases in 25-30% of cases and treatment strategy is not curative in these cases [1]. Therefore, new treatment options are urgently needed. Since LC tumors overexpressed the vascular endothelial growth factor (VEGF) and the VEGF receptor (VEGFR) subtypes [2], small molecule tyrosine kinase inhibitors (TKIs) could be taken into account for this disease.

In particular, axitinib (AXI), a small TKI, is a potent and selective inhibitor of VEGFR 1, 2, and 3 approved by FDA in 2012 for the treatment of patients with metastatic renal cell carcinoma [3, 4].

OBJECTIVE

In this preclinical study, we investigated the antitumor activity of AXI in human LC cell lines (NCI-H727, UMC-11 and NCI-H835) and its effect on tumor-induced angiogenesis in zebrafish xenografts implanted with these neuroendocrine tumors (NETs).

METHODS

In vitro and in vivo preclinical models: our platform for analysis of AXI effect in lung NETs.

Anti-tumoral activity of axitinib in preclinical models of lung carcinoids: Chronicle of a Death Foretold

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Evaluation of cell morphology

Figure 1: Effects of AXI on cell morphology in lung NET cell lines after 6 days of incubation. Images were detected by 20x objective – Lumen after staining with Hoechst (A) and Green cell tracker image descriptors (area and circularity) were detected by image software after (B) pixel unit. Area analysis analysis detected by Hoechst and area whole cell analysis detected by Green cell tracker treatment stage for single cell. Nuclear circularity assay is based on the area and perimeter of the nucleus. Circularity has a maximum value of 1 and decreases as the nucleus shape becomes increasingly convoluted. Images represent the mean ± SEM of at least 3 independent experiments. For statistical analysis GraphPad Prism 5.0 (GraphPad Software, San Diego, CA) was used and unpaired Student’s t test was chosen. * p < 0.05, ** p < 0.01, *** p < 0.001. AXI: 0.25 µM of standard error of the mean, CTR: control.

Evaluation of DNA damage and cell death

Figure 5: Effects of AXI on DNA damage and cell death after 6 days of incubation. Preliminary representative Western blot images for the expression of key proteins of DNA damage, CHK2, CHK1 and pCHK1, after 6 days of incubation with AXI in lung NET cells. AXI was used as a loading control. For IHC analysis, cells were stained with Annexin V-IR. The proportions of NCI-H727 (A), UMC-11 (B), NCI-H835 (C) in necrosis, late apoptosis and early apoptosis are expressed as percentage compared with untreated CTR and represent the mean ± SEM of at least 3 independent experiments. For statistical analysis GraphPad Prism 5.0 (GraphPad Software, San Diego, CA) was used and unpaired Student’s t test was chosen. * p < 0.05. *** p < 0.001. Preliminary representative Western blot images for the expression of the key proteins of cell death processes such as total and cleaved PARP, the total and cleaved caspase 3 after 6-days of incubation with AXI in lung NET cells. AXI was used as a loading control.

Conclusion

Axitinib exerts a prominent antitumor activity modulated by cell cycle arrest, induction of selective cell death mechanisms and inhibition in tumor-induced angiogenesis in LC preclinical models, suggesting a potential therapeutic application in patients with advanced LC tumors.

References