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

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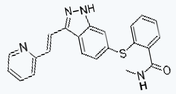
INTRODUCTION

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 in account for this disease.

In particular**, axitinib (AXI)**,



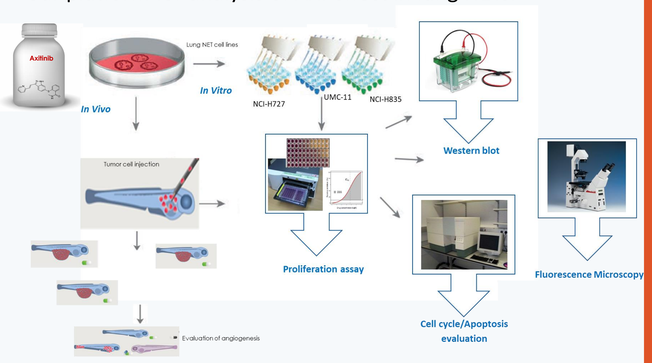
a small TKI, is a potent and selective inhibitor of VEGFR 1, 2, and 3 approved by FDA in 2012 for the treatment of patients with metastatic renal cell carcinoma [3, 4].

OBJECTIVE

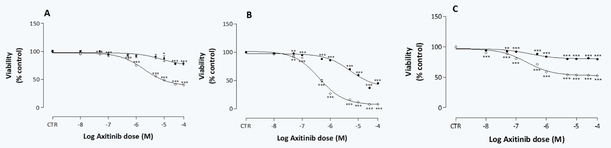
In this preclinical study, we investigated the antitumor activity of AXI in human LC cell lines (NCI-H727, UMC-11 and NCI-H835) and its effect on tumor-induced angiogenesis in zebrafish xenografts implanted with these neuroendocrine tumors (NET) cells.

METHODS

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



Cell viability



Axitinib	EC_{50} (M)		maximal inhibition	
	3D	6D	3D	6D
NCI-H727	$8{,}6 * 10^{-6}$	$1{,}9 * 10^{-6}$	-27%	-61%
UMC-11	$5 * 10^{-6}$	$4 * 10^{-7}$	-63%	-93 %
NCI-H835	$2{,}8 * 10^{-7}$	$2{,}2 * 10^{-7}$	-20%	-47%

Figure 1 : **Effect of AXI on cell viability in NCI-H727 (A), UMC-11 (B) and NCI-H835 (C) as measured by MTT assay at 3 (●) and 6 ( ) days**. For statistical analysis GraphPad Prism 5.0 (GraphPad Software, San Diego, CA) was used. The comparative statistical evaluation among groups was performed by the ANOVA test. When significant differences were found, the Newman–Keuls test was used for comparison between groups. The values were reported as the mean ± S.E.M. from at least three independent experiments. *p<0.05; ** p<0.01; *** p<0.001.

Evaluation of cell morphology

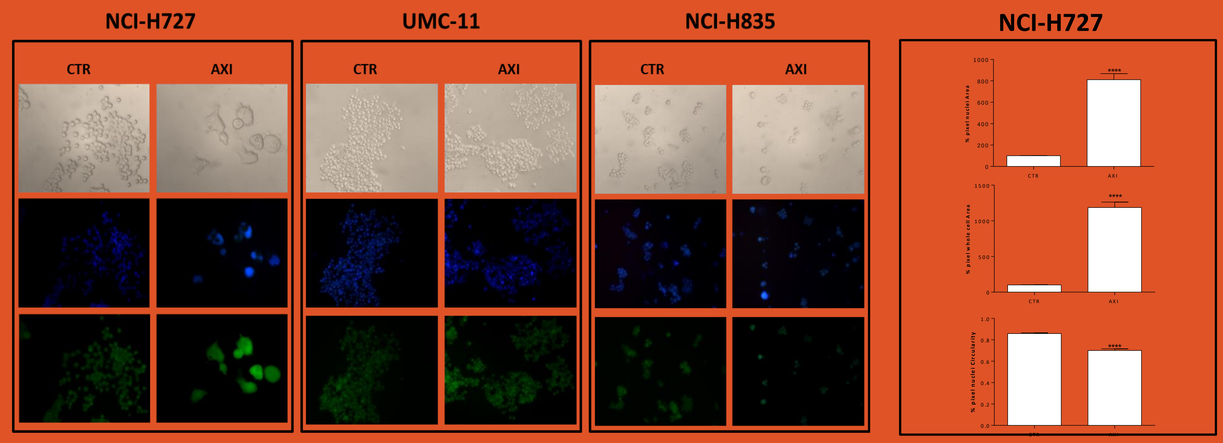


Figure 3 : **Effects of AXI on cell morphology in lung NET cell lines after 6 days of incubation.** Images were detected by 20X objective – Leica after staining with Hoechst (blue) and Green cell tracker. Shape descriptors (area and circularity) were detected by ImageJ software after image calibration to pixel unit. Area nuclei analysis detected by Hoechst and area whole cell analysis detected by Green cell tracker fluorescent signal for single cell. Nuclear circularity analysis is based on the area and perimeter of the nucleus. Circularity has a maximum value of 1 and diminishes as the nuclear shape becomes increasingly convoluted. Values 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: axitinib; SEM: standard error of the mean; CTR: control.

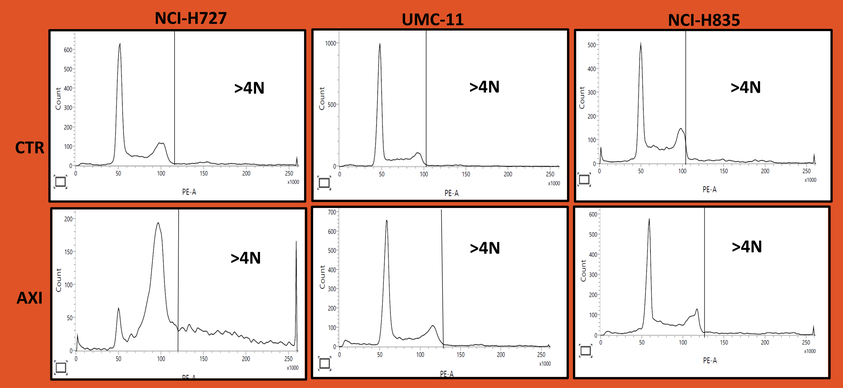


Figure 4: **Representative flow cytometry with DNA contents of lung NET cells after treatment with AXI at 6 days.**

Evaluation of DNA damage and cell death

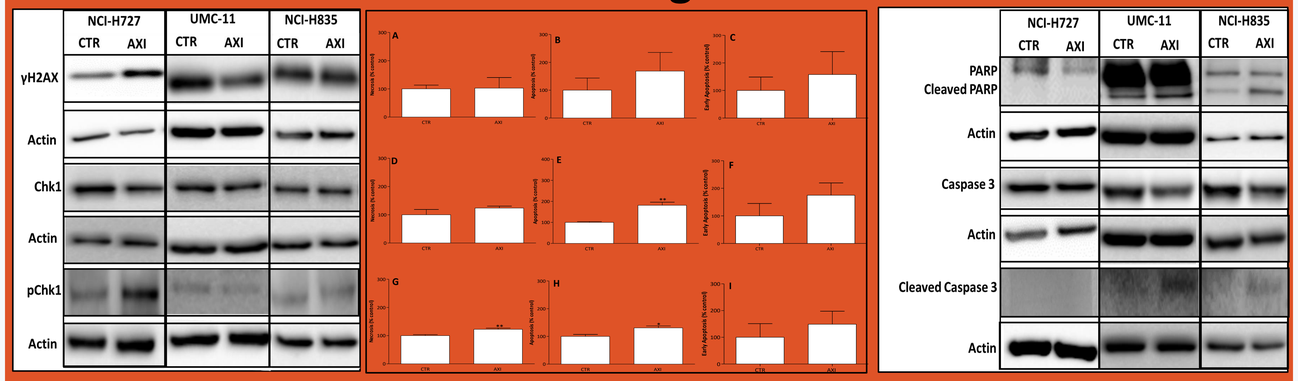


Figure 5 : **Effects of AXI on DNA damage and cell death after 6 days of incubation.** Preliminary representative Western blot images for the expression of key proteins of DNA damage, γH2AX, Chk1 and pChk1, after 6 days of incubation with AXI in lung NET cells. Actin was used as a loading control. For FACS analysis, cells were stained with Annexin V/PI. The proportions of NCI-H727 (A-C), UMC-11 (D-F), NCI-H835 (G-I) 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<0.01. 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. Actin was used as a loading control.

Evaluation of cell cycle

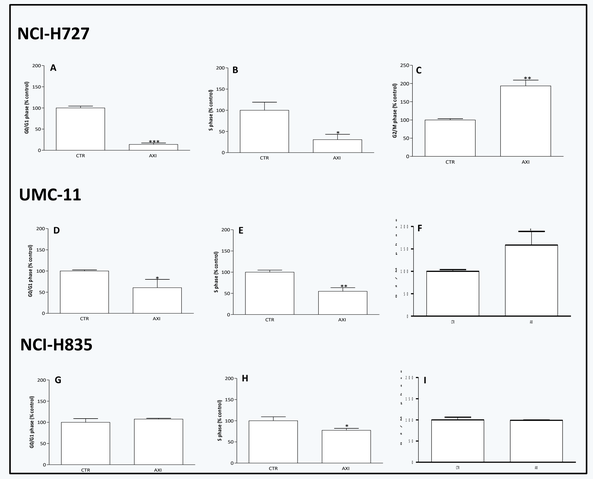
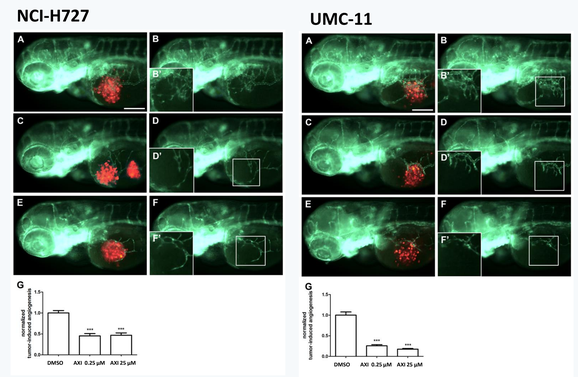


Figure 2 : **Effects of AXI on cell cycle in NCI-H727 (A-C), UMC-11 (D-F), NCI-H835 (G-I) after 6 days of incubation.** Cell cycle distribution is expressed as percentage of cells in G0/G1, S, and G2/M phases compared to vehicle control (CTR) cells. CTR values have been set to 100%.For statistical analysis 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.

Evaluation of tumor-induced angiogenesis



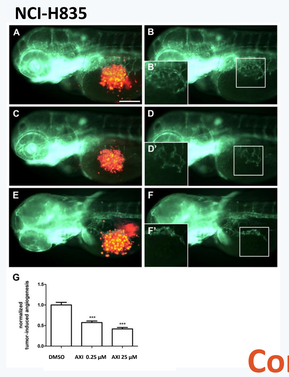


Figure 6: **Effect of AXI on NCI-H727, UMC-11 and NCI-H835-induced angiogenesis in zebrafish embryos.** Representative epifluorescence images of *Tg(fli1:EGFP)y1* zebrafish embryos, implanted with lung NET cells and treated with DMSO (A, B and B'), as control, and 0.25 (C, D and D') and 2.5 µM (E, F and F') AXI. The red channel, corresponding to carcinoid NET cells, was omitted in panels B, B', D, D', F and F' to highlight the tumor-induced microvascular network. Digital magnifications of graft region are showed in white-boxed regions B', D' and F'. Panel G showed the quantification of tumor-induced angiogenesis in carcinoid NET cells injected embryos after 24 hours of AXI treatments at 0.25 and 2.5 µM (G). Control (DMSO) values have been set to 1.0. Graphed values represent the mean ± S.E.M. ***p<0.001 vs DMSO. All images are oriented so that rostral is to the left and dorsal is at the top. Scale bar, 100 µm.

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

[1] Iyoda A et al. 2020
[2] Telega A et al. 2012
[3] Kelly RJ et al. 2009
[4]Parekh H et al. 2016