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Natural Compound Verrucarin A Potentiates the Anticancer Effect of Etoposide in NET Cell Lines

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BACKGROUND: First line chemotherapy treatment options for advanced neuroendocrine tumors (NETs) are limited and etoposide remains the standard NET regimen for over 40 years. Etoposide acts by stabilizing a normally transient DNA-topoisomerase II complex, thus increasing the concentration of double-stranded DNA breaks and triggering mutagenic and cell death pathways. Drug resistance, low bioavailability, and systemic toxicity of etoposide are the major obsta_cles in NET treatment. To improve responsiveness to etoposide we propose to combine this drug with a highly potent natural compound - verrucarin A (VC-A), which inhibits NET prosurvival pathway Akt/NF-_B/mTOR.

METHODS: NET cell lines (BON, pancreatic NET and H727, pulmonary NET) were treated with VC-A or etoposide for 72 hours and an MTT assay was used to determine IC50 values. Flow cytometric analysis of bromodeoxyuridine and 7-AAD staining were used to determine the effects on cell cycle of VC-A, etoposide, and combination treatments. Western blot analysis was performed to determine the effects on phospho-AKT and phospho-mTOR signaling.

RESULTS: BON and H727 cell lines demonstrated high sensitivity to VC-A. Pulmonary fibroblasts (WI-38) and normal thyroid cells (NThyori) with doubling times shorter or comparable to BON and H727 cells demonstrated a decreased sensitivity to VC-A. Etoposide reduced the number of cells in S-phase and increased the percentage of cells in the G2/M phase of the cell cycle. Co-treatment with VC-A decreased the number of cells in active cell cycle in a dose-dependent manner compared to etoposide alone. Moreover, a synergistic effect was observed between etoposide and VC-A. Western blot analysis showed a

decrease in AKT/mTOR phosphorylation.

CONCLUSION: Verrucarin A and etoposide synergistically inhibit the growth of NET cell lines. Moreover, the combined therapies could avoid the possible drug resistance developed by a single agent. Specifically targeting NETs with VC-A and etoposide within exosomes could improve patient outcomes while limiting toxicities.

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