STRONG CHILDREN'S RESEARCH CENTER

Summer 2016 Research Scholar

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ABSTRACT

Title: ***Discovery of Comp-A: A MEK Inhibitor for Treatment of Clear Cell Ovarian Carcinoma***

Background: Clear cell ovarian carcinoma (CCOC), a subtype of epithelial ovarian cancer (EOC), is the most lethal ovarian malignancy. Patients with CCOC currently receive therapies intended for EOC more broadly, which tend to fail, necessitating the development of CCOC specific therapies. Aberrant Ras/Raf/MEK/ERK signaling promotes onset, progression and chemoresistance among various cancer-types, including EOC.

Objective: To explore the effects of Comp-A, a novel MEK inhibitor developed in our laboratory, on ERK regulation, cell proliferation, and cell survival in in-vitro CCOC models.

Methods: Two independent kinase panel inhibition studies were conducted to determine kinase inhibition potency and selectivity. CCOC (OVMANA and OVTOKO) cells were cultured in RPMI media supplemented with 10% FBS and 1% antibiotic in a humidified incubator with 5% CO_2. MTS Cell Proliferation Colorimetric Assay and BrdU Cell Proliferation Assay were used to evaluate the effect of Comp-A on cell proliferation and cytotoxicity. All drug treatments were run in triplicate. Regulation of ERK (immediate downstream substrate of MEK) in response to Comp-A treatment was assessed by Western blotting. The presence of apoptotic cells and the effect of Comp-A on cell-cycle progression were determined by cell cycle analysis. OVMANA cells were treated with or without Comp-A, fixed with 70% ethanol, and stained with PI/RNase solution. DNA content was quantified using flow cytometry.

Results: Comp-A inhibited MEK1 (IC50: 5.91μM) and MEK2 (IC50: 2.27μM) in an in-vitro kinase screening assay. In another independent vendor study, Comp-A showed IC50 of 30 nM against MEK 1/2 kinase. Treatment with Comp-A inhibited cell proliferation of CCOC cells, as demonstrated by the BrdU and MTS Assays. Only OVMANA cells exhibited inhibition after 48 hrs., but both OVMANA and OVTOKO cells exhibited dose-dependent inhibition of cell proliferation after 72 hrs. Comp-A also inhibited the activation of the pro-survival ERK1/2 pathway in both OVMANA and OVTOKO cells. Comp-A was found to reduce ERK1/2 activation induced by cisplatin, a chemotherapeutic agent commonly used in treatment of EOC. Comp-A does not cause cellular apoptosis in CCOC cells. Cells treated with DMSO or 5μM Comp-A were mostly present in G0/G1 phase, as determined using flow cytometry.

Conclusion: Comp-A is a low micromolar, selective inhibitor of MEK1 and MEK2. Treatment with Comp-A inhibits proliferation of CCOC cells, with OVMANA cells exhibiting greater inhibition than OVTOKO cells. Comp-A results in downregulation of pro-survival ERK1/2 phosphorylation in CCOC cells within 1 hr. of treatment. Cisplatin treatment promotes pro-survival ERK1/2 phosphorylation, which may result in emergence of resistance. Comp-A may potentially be able to counteract this route of resistance emergence. Further studies are needed to explore the effects of combining these drugs. Preliminary studies indicate that Comp-A may invoke an apoptosis independent and cell cycle independent mechanism of action. Further studies are needed to establish the mechanism of action of Comp-A. Determination of anti-tumor efficacy and MEK1/MEK2 receptor engagement in animal models of CCOC are proposed.