Depression of mitochondrial bioenergetics is a potent death stimulus in the ovarian cancer stem cells

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BACKGROUND

- Cancer stem cells represent the cell population responsible for tumor initiation and progression.
- In ovarian cancer, the CD44+/MyD88+ epithelial ovarian cancer (EOC) stem cells represent the chemo-resistant population.
- We previously showed that the novel isoflavone derivative, NV-128, can induce caspase-independent cell death in the EOC stem cells.
- We demonstrate in this study that NV-128 is able to depress mitochondrial function leading to the activation of two independent cell death pathways in these chemo-resistant cells.

METHODS

A panel of CD44+/MyD88+ EOC stem cells was treated with NV-128 (10μg/ml). Inhibitory studies were done using the specific MEK inhibitor, U0126 (10μM), or the ROS scavenger, MnTBAP (500μM). Mitochondrial function was assess-ed using the JC1 dye, MitoSox dye, and ApoSENSOR ADP/ATP kit. Pro-tein levels were determined using Western Blot.

KEY FINDINGS

(1) NV-128 is able to elevate mitochondrial superoxide levels and inhibit ATP production in the EOC stem cells.

(2) Superoxide production acti-vates the ERK/Bax axis, which results in loss of mitochondrial membrane potential.

(3) Loss of ATP activates AMPKα1, leading to mTOR inhibition.

(4) Depression of mitochondrial function is associated with loss of Cox-IV.

Figure 1. Molecular effects of NV-128 treatment. EOC stem cells were treated with 10μg/ml NV-128 at designated times. Treatment resulted in increase mitochondrial superoxide (A), decrease in ATP production (B), decrease in Cox-IV and pS6 kinase, and activation of ERK and AMPKα1 (C). # $p < 0.0001$ compared to control.

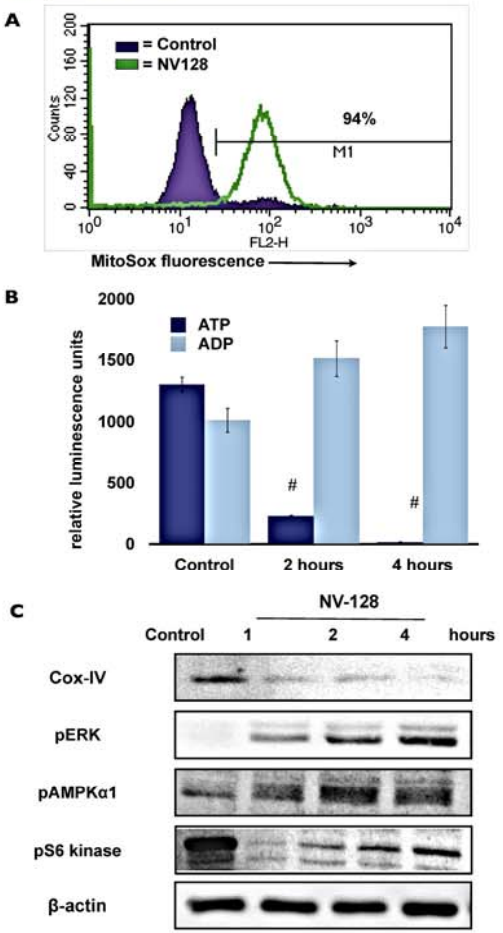
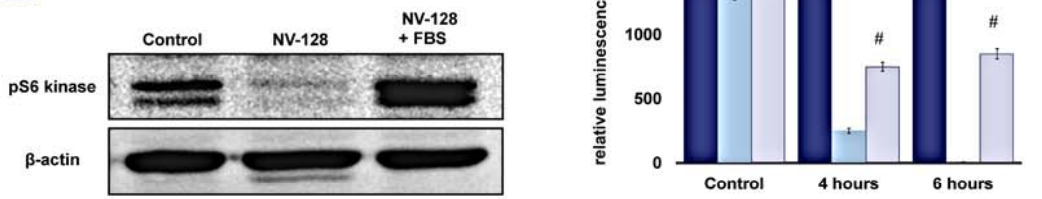
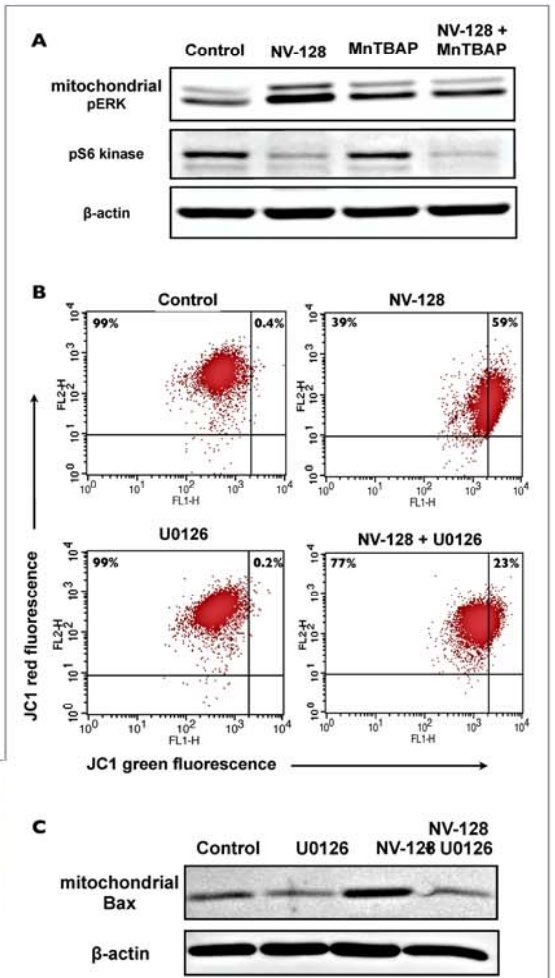


Figure 2. NV-128-induced ROS production leads to ERK activation and loss of mitochondrial membrane potential. (A) EOC stem cells were treated with NV-128 in the presence or absence of the ROS scavenger, MnTBAP. MnTBAP inhibited NV-128-induced activation of mitochondrial ERK, but not NV-128 induced decrease in pS6 kinase. (B, C) EOC stem cells were treated with NV-128 in the presence or absence of the MEK inhibitor, U0126. U0126 inhibited NV-128-induced loss of mitochondrial membrane potential and mitochondrial translocation of Bax.

Figure 3. NV-128-induced loss of ATP leads to mTOR inhibition. EOC stem cells were treated with NV-128 in the presence of 10% FBS. Note the abrogation of ATP loss and pS6 kinase inhibition with FBS. # $p < 0.001$ compared to NV-128 alone.





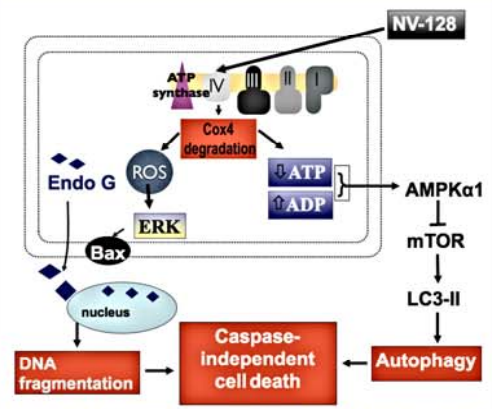


Figure 4. Proposed model for NV-128-induced cell death in EOC stem cells. By targeting the mitochondria, NV-128 activates two independ-ent cell death pathways. Degradation of Cox-IV leads to ATP loss and increase mitochondrial ROS. ATP loss leads to inhibition of mTOR pathway and autophagic cell death. ROS activates the ERK/Bax axis leading to loss of mitochondrial membrane potential and EndoG-dependent DNA fragmentation.

CONCLUSION

- Depression of mitochondrial function is a potent stimulus to induce cell death in the EOC stem cells and opens new venues for treating ovarian cancer patients.
- Novel NV-128 derivatives such as NV-344, are being developed with improved potency.