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14. ABSTRACT

ERK signaling pathway has long been suggested as a therapeutic target for ovarian cancer progression and metastasis. In our previous studies, we showed that 1) high Erk activity is sensitive to the elevation of intracellular cAMP concentration; and 2) agents elevating cellular cAMP suppresses growth of aggressive ovarian cancer cells. This proposal is sought to 1) understand molecular mechanisms associated with forskolin/PDE2 inhibitor-induced apoptosis of aggressive ovarian cancer cells and 2) to evaluate the translation value of treating aggressive ovarian cancer cells with forskolin and PDE2 inhibitor in an intraperitoneal xenograft model. In first year of the funding, we showed that knockdown of PDE2A rendered ovarian cancer cells susceptible for forskolin-induced cell growth inhibition/apoptosis. We further showed that combined use of forskolin and Bay60-7550 (PDE2 inhibitor) downregulates the levels of Bcl2, survivin and phosphorylated Akt whereas induces the expression of Bim1. The effect of forskolin/Bay60-7550 is clearly mediated by PKA because PKA inhibitor H89 abolished growth inhibition caused by forskolin/Bay60-7550. Results from our first year study elucidate molecular mechanism underlying forskolin/PDE2 inhibitor-led ovarian cancer cell growth inhibition/apoptosis and built basis of testing forskolin/Bay60-7550 in ovarian cancer models.

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Introduction

Ovarian cancer has the highest mortality rate among gynecological cancers. Due to the lack of symptoms with early stage disease and no effective screening strategies, ovarian tumors have become metastatic at the time of clinical diagnosis in 75% of ovarian cancer patients. Unfortunately, advances in surgical techniques and chemotherapy have not significantly improved the survival of ovarian cancer patients in past several decades. Therefore, the novel therapeutic approaches are urgently needed for treating metastatic ovarian cancers.

ERK signaling pathway plays a critical role in the survival and proliferation of ovarian cancer cells. It is thus expected that approaches interfering with ERK signaling pathway will lead to the suppression of ovarian cancer progression and metastasis. In our previous studies, we showed that 1) Erk activity is higher in aggressive ovarian cancer cells and is required for their survival; 2) high Erk activity is sensitive to the elevation of intracellular cAMP concentration; 3) combined use of forskolin and PDE2 inhibitor induces apoptosis of aggressive ovarian cancer cells. This proposal is 1) sought to understand molecular mechanisms associated with forskolin/PDE2 inhibitor-induced apoptosis of aggressive ovarian cancer cells and 2) to evaluate the translation value of treating aggressive ovarian cancer cells with forskolin and PDE2 inhibitor in an intraperitoneal xenograft model. In first year of the funding, we showed that knockdown of PDE2A rendered ovarian cancer cells susceptible for forskolin-induced cell growth inhibition/apoptosis. We further showed that combined use of forskolin and Bay60-7550 (PDE2 inhibitor) downregulates the levels of Bcl2, survivin and phosphorylated Akt whereas induces the expression of Bim1. The effect of forskolin/Bay60-7550 is clearly mediated by PKA because PKA inhibitor H89 abolished growth inhibition caused by forskolin/Bay60-7550. Results from our first year study elucidate molecular mechanism underlying forskolin/PDE2 inhibitor-led ovarian cancer cell growth inhibition/apoptosis and thus built basis of testing forskolin/Bay60-7550 in ovarian cancer models.

Body

Tasks of first year of this proposal are 1) Examine the effect of PDE1C and PDE2A-specific siRNAs on ovarian cancer cell growth inhibition/apoptosis (months 1-4); 2) Examine the effect of PDE1 and PDE2 inhibitors (8-methoxymethyl-IBMX and Bay60-7550) on ovarian cancer cell growth inhibition/apoptosis (months 5-8); 3) Determine potential involvement of PKA in PDE1/2 inhibitor-led ovarian cancer cell growth inhibition/apoptosis.

Task 1: We introduced PDE1C or PDE2A siRNAs into ovarian cancer ES2 and OVCAR5 cells and measured their effect on cell growth. Only slightly growth inhibitory effect was seen with PDE2A siRNA in aggressive ovarian cancer cells. To determine if lack of growth inhibition by PDE siRNA is caused by poor accumulation of cellular cAMP in these cells, we treated these cells with 10μM forskolin two days after siRNA transfection. MTT assay showed that forskolin suppressed over 60% of cell growth in cells with PDE2A-knockdown but not PDE1C-knockdown in 3-day growth period. Silencing both PDE1C and PDE2A did not make forskolin more growth inhibitory. Moreover, forskolin alone only marginally inhibited growth of cells treated with control siRNA (Fig.1). Similar results were also generated with another four ovarian cancer cells lines (HEY, OCC1, OVCAR8 and SK-OV3) (data not shown). These results suggest that forskolin can effectively ovarian cancer cells growth when PDE2A is blocked.

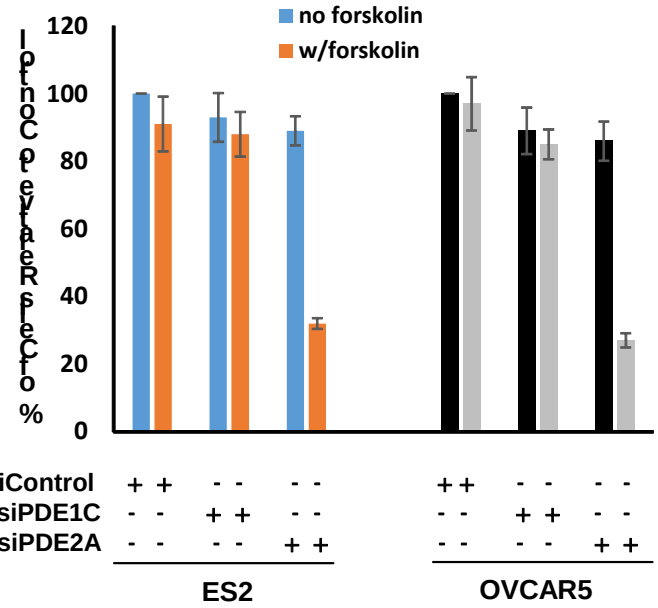

Figure 1. Forskolin suppresses growth of ovarian cancer cells with PDE2A-knockdown. ES2 and OVCAR5 cells were transfected with control siRNA or siRNA pool for PDE1C or PDE2A for 2 days and then treated with 10μM forskolin or left untreated for 3 days. Cell growth was evaluated by MTT assay and % of cells relative to control for untreated siRNA control was taken as 100% for each cell line.

Task 2: ES2 and OVCAR5 cells were first treated with PDE2 inhibitor Bay60-7550 or PDE1 inhibitor 8-methoxymethyl-IBMX for 2 h followed by treatment of forskolin for 3 days. MTT assay clearly showed that Bay60-7550, but not 8-methoxymethyl-IBMX rendered cells responding to forskolin for growth inhibition (Fig.2A). Similar results were also generated with HEY, OCC1, OVCAR8 and SK-OV3 cells (data not shown). To determine whether growth inhibition was caused by cell death, we performed Annexin V/PI-staining on OVCAR5 cells treated with or without forskolin/Bay60-7550. Flow cytometry revealed a dramatic increase in apoptotic cells (Fig.2B). To elucidate molecular mechanism associated with forskolin/PDE2 inhibitor-induced apoptosis, we collected cell lysates from untreated OVCAR5 cells and OVCAR5 cells treated with forskolin/Bay60-7550 or forskolin/EHNA (another PDE2 inhibitor). Western blotting showed combined treatment but none of them alone led to reduction in the

abundance of Bcl2, survivin and phosphor-Akt while increased the level of Bim1 (Fig.2C). These results suggest that forskolin/PDE2 inhibitor treatment suppresses ovarian cancer cell

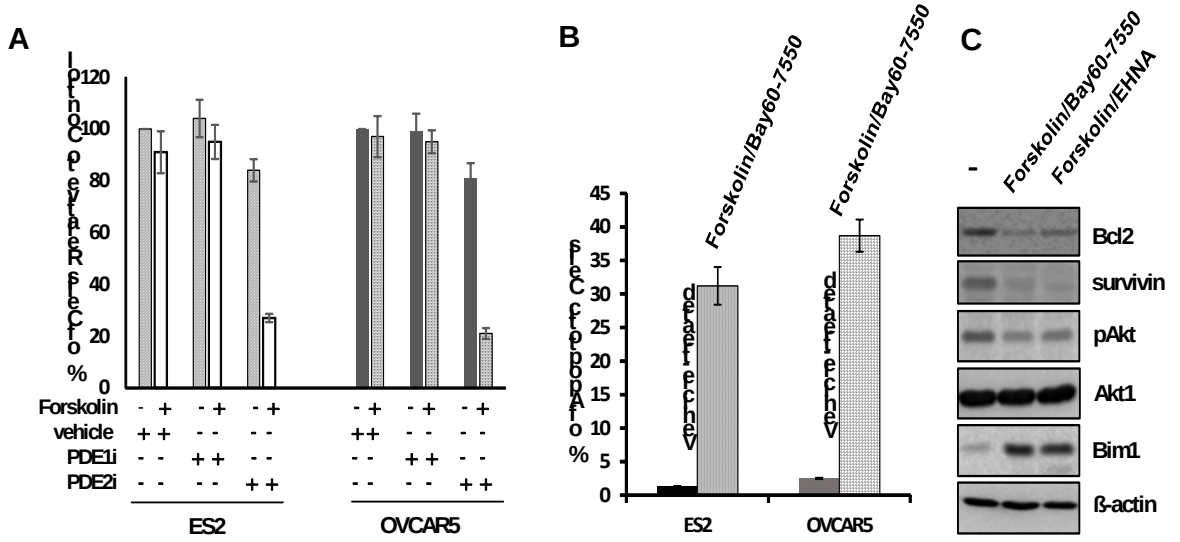

Figure 2. Forskolin/PDE2 inhibitor suppresses growth of ovarian cancer cells. ***A.*** ES2 and OVCAR5 cells were pretreated with PDE1 or PDE2 inhibitor (8-methoxymethyl-IBMX or Bay60-7550) for 2 h and then treated with 10μM forskolin or left untreated for 3 days. Cell growth was evaluated by MTT assay. % of cells relative to control is for cells treated with vehicle only and was taken as 100%. ***B.*** Vehicle or forskolin/Bay60-7550-treated cells were subjected to Annexin V-labeled flow cytometry. Apoptotic cells were cells positive for Annexin V staining. ***C.*** OVCAR5 cells were treated with forskolin/Bay60-7550, forskolin/EHNA or left untreated for 2 days, then lysed and cell lysates were subjected to western blotting to detect Bcl2, survivin, phosphor-Akt and Bim1. ß-actin was also determined as a loading control.

growth by inducing cell death through the inhibition of Bcl2/survivin/phosphor-Akt/and upregulation of Bim1.

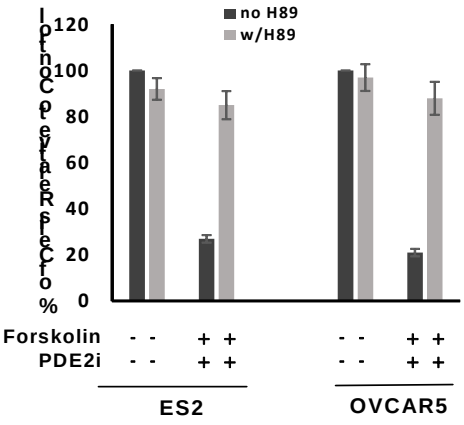

Figure 3. Forskolin/Bay60-7550 suppresses ovarian cancer cell growth in a PKA-dependent mechanism. ES2 and OVCAR5 cells were pretreated with PKA inhibitor H89 for 2 hr followed by the treatment of forskolin/Bay60-7550 for 3 days. MTT assay was performed to evaluate cell growth. % of cells relative to control for untreated cells was taken as 100% for each cell line.

Task 3: To determine the potential role of PKA in forskolin/Bay60-7550-led ovarian cancer cell growth inhibition/apoptosis, we pretreated ES2 and OVCAR5 cells with PKA inhibitor H89 for 2 h prior to the treatment of forskolin/Bay60-7550. MTT assay showed that H89 abolished at least 80% of forskolin/Bay60-7550-caused growth inhibition (Fig.3), thus confirming that PKA as the mediator for forskolin/Bay60-7550-led growth inhibitory effect in ovarian cancer cells.

Key Research Accomplishment

We found that 1) Inhibiting PDE2 makes ovarian cancer cells susceptible to forskolin-induced growth inhibition/apoptosis. 2) Forskolin/Bay60-7550-caused ovarian cancer cell growth inhibition/apoptosis is PKA-dependent.

Reportable Outcomes

Our study has built the basis for further testing forskolin/Bay60-7550 in murine tumor model for their anti-tumor effectiveness.

References

None

Appendices

N/A