Effect of actinomycin d and pma on cell line 769-p, a line of human renal cell ca...

Health & Medicine, Cancer



Abstract

Renal cell carcinoma is a devastating disease that oftentimes results in a poor prognosis because there are few effective chemotherapeutic options. The renal carcinoma cell line 769-P is an ideal in vitro model for investigating novel drug treatments for renal cell carcinoma. Two drugs that may potentially reduce renal cell carcinoma proliferation are Actinomycin D and phorbol 12-myristate 13 acetate (PMA). Both of these drugs have been shown to inhibit cell proliferation in other in vitro models of cancer. This study examined the effects of Actinomycin D and PMA treatment on the proliferation of 769-P cells. Cell viability studies indicate that both drugs successfully reduce the number of 769-P cells. Apoptotic assays demonstrated a dose dependent increase in apoptotic 769-P cells after Actinomycin D treatment. Both drugs also reduce the ability of 769-P cells to grow in soft agar indicating a reduction in the cells' ability for anchorage independent growth which is clear marker for metastatic growth. The results of this study suggest that Actinomycin D and PMA may be effective novel chemotherapeutic agents in the fight against renal cell carcinoma.

Introduction

Renal cell carcinoma is a lethal cancer that accounts for about 92% of all kidney cancers in the United States. 1 Initially it affects cells lining the tubules of the kidney. 2 Renal cell carcinoma is the tenth leading cause of cancer death among men. It is estimated that nearly 65, 000 new cases will be diagnosed and nearly 14, 000 people will die of renal cell carcinoma in 2012. 3 The 5-year survival rate for localized renal cell carcinoma is about it is crucial that new therapeutic drugs are explored.

70%, however, about 30% of renal cell carcinoma cases are diagnosed with metastatic forms of the disease and the 5 year survival rate for such cases is only about 10%1. Currently, surgery is the primary treatment of renal cell carcinoma. Chemotherapy is not very effective at treating this disease and this is largely thought to be linked to multidrug-resistant P-glycoproteins. 3-4 As there is no effective chemotherapeutic treatment of renal cell carcinoma,

The renal cell line 769-P originated from cells taken from the resected kidney of a 63-year old male Caucasian diagnosed with renal cell carcinoma and is a stable cell line that can be used to study drug therapies targeting the disease. 5 This cell line has been used in a number of renal cell carcinoma studies and cells of this line are thought to be a good in vitro model of this disease. 6-7

Two drugs that may be effective against renal cell carcinoma are Actinomycin D and phorbol 12-myristate 13 acetate (PMA). Actinomycin D is a transcription inhibitor which intercalates with DNA to inhibit transcription, thus blunting cell proliferation. 8 Actinomycin D has effectively reduced cells in models of other cancers including lymphocytic leukemia and gestational trophoblastic neoplasia. 9-10 PMA is a tumor promoting phorbol ester that is known to modulate protein kinase C activity and has been shown in a number of cancer studies to have apoptotic properties. 11-12 As the incidence of renal cell carcinoma continues to rise1, it is increasingly important to explore new compounds as possible therapies for this type of cancer. As such, in this study, we aim to investigate the effect of two well know chemotherapeutic agents, Actinomycin D and PMA, on the renal cell carcinoma line 769-P.

Methods

Cell Lines and Cell Cultures 769-P cells originated from clear renal cell adenocarcinoma were cultured. (these details were not provided in the procedures so you can add details or delete this section)

Cell Viability Assay

Apoptosis Assay

Soft Agar Assay

Results-I did not include the figures in the text and just kept them in the PP file so you can add them as you feel fit. I did refer to them in the text.

Cell Viability Assay

The ED50 of Actinomycin D is about 100 ng/ml (Figure 1) while the ED50 of PMA is about 0. 6 ng/ml (Figure 2). Treatment of 679-P cells with doses of Actinomycin D higher than 1. 5 ng/ml resulted in significant reduction in viable cells in compared to untreated cells (control). Treatment with 1. 23 ng/ml, 11. 11 ng/ml and 33. 33 ng/ml (NOTE that in the results that you provided the doses for PMA are first given in ng/ml units and then nmol unitswhich is correct?) doses of PMA significantly reduced the number of viable 679-P cells compared to untreated 679-P cells (control) (Table 1). Apoptosis Assay-I am still a little confused because you mentioned in the last email message you wrote " the top is the cell line 769P : we used different

concentration : We start with 10, 000 cells; for the Actinomycin D : we start with 123", however, in the figure you describe the 10, 000 and 123 as dosages and not cell numbers. As such, I am just going to describe the results as I see them qualitatively. I am not sure if you quantified the cells. If so, you can add those results here too.

Hoechst/Propidium lodide staining of the cells treated with Actinomycin D revealed that the number of Hoechst positive cells decreased while the number of Hoechst/Propidium lodide stained cells increased. At low concentrations of Actinomycin D, cells are abundant, however, as the dose increases, cells become more sparse (Figure 2).

Soft Agar Assay

Anchorage-independent colony formation of 679-P cells was determined by soft agar assay. Cells treated with 1: 3 and 1: 8 dilutions of PMA resulted in nearly half the colony formation compared to untreated 679-P cells (control) (Table 2). Cell suspensions treated with actinomycin experienced a dose dependent reduction in colony formation compared to control such that control treatment resulted in . 63 % colony formation, 370 ng/ml Actinomycin resulted in . 40% colony formation and 10, 000 ng/ml resulted in no colony formation (Table 3).

Discussion

In this study, we investigated the effects of Actinomycin D and PMA on the proliferation of 769-P cells, a cell line of renal cell carcinoma. We found that both Actinomycin D and PMA treatment significantly decreased tumor cell viability, increased apoptosis and reduced anchorage independent tumor cell growth. These findings indicate that Actinomycin and PMA may be effective therapeutic drugs against renal cell carcinoma, a form of cancer that currently has no efficacious chemotherapeutic treatment options. Actinomycin D is a well-known polypeptide antibiotic and was one of the first antibiotics to be used as a chemotherapeutic agent against cancer. 13 Actinomycin D halts cell proliferation by binding to DNA, inhibiting RNA synthesis and essentially stabilizing DNA. The end result of this is that cell proliferation is reduced. 8, 13 Thus, it is no surprise that, in this study, 679-P cells treated with Actinomycin D even at low concentrations experienced significantly less cell proliferation compared to untreated control 679-P cells. Actinomycin D is a very effective treatment of pediatric cancers and is particular crucial in treating Wilms' tumors. Actinomycin D was crucial in increasing the survival rate of Wilms' tumor to 80-90% while causing relatively few negative side effects. 14-15

While Actinomycin D is well known in the cancer world, far less is known about the anti-cancer effects of PMA. PMA is a phorbol ester that is wellknown for its tumor promoting properties. PMA activates protein kinases C which, in turn, modulates the activity of many cell signaling pathways downstream including the MEK/ERK pathway. Recently, the activation of the MEK/ERK pathway has been shown to be beneficial to successfully targeting chemo resistant ovarian cancer cells. 12

The data in this study show that both Actinomycin D and PMA are effective at reducing 679-P cells, however, it appears that 679-P is more sensitive to Actinomycin D. At all treatment doses, Actinomycin D treated cells were significantly less viable than untreated controls, whereas only at a few doses was the cell viability significantly less in PMA treated cells compared to untreated controls. Actinomycin was also more efficient at reducing colony formation in the soft agar assay and, in fact, at its highest dose, Actinomycin D completely inhibited the formation of new cell colonies. One possible explanation for this observation is that, in this study, the Actinomycin D concentrations were much higher than the PMA concentrations. Follow up studies investigating this possibility would be enlightening. Future studies investigating whether combined treatment of Actinomycin D and PMA would be even more effective in targeting 679-P cells would be interesting, as well. Note-You might want to add a sentence or two highlighting possible sources of error in this studyl could not identify any with the information you provided.

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