Abstract
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Antitumor activity of PBI-1737 in xenograft human prostate (PC-3) cancer by inhibition of cell adhesion and migration

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Abstract

Cell adhesion, proteolytic degradation of extracellular matrix and cell migration are interrelated processes responsible for the invasion and metastasis of cancer. In prostate cancer, androgen-independence and bone metastasis are lethal complications in patients. PBI-1737 is a low molecular weight, orally active molecule that inhibits invasion and adhesion of androgen-independent human PC-3 cells. PBI-1737 demonstrated an inhibition of the EGF-induced PC-3 invasion in a two-dimension cell mobility assay. Furthermore, the addition of different concentrations of PBI-1737 induces in a dose dependent manner an inhibition of PC-3 cells adhesion to laminin, matrigel and collagen. The antitumor efficacy of oral administration of PBI-1737 with or without combination of cyclophosphamide on xenograft human prostate PC-3 tumor was also studied. Oral administration of PBI-1737 (50 mg/kg) induces a significant ($p < 0.05$) inhibition of tumor volume with a T/C between 14% to 40%. Cyclophosphamide (i.p., 100 mg/kg, once a week for 4 weeks) induces a significant inhibition ($p < 0.05$) of tumor volume with a T/C between 1% to 39%. Mice treated with the combination of cyclophosphamide and oral administration of PBI-1737 demonstrated a significant ($p < 0.01$) inhibition of tumor volume with a T/C between 1% to 40% accompanied with tumor regression. These *in vivo* and *in vitro* data indicate that oral administration of PBI-1737 demonstrated significant antitumor activity via an inhibition of cell adhesion and migration of human prostate PC-3 cancer cells. A synergistic effect (regression of the tumor) is also observed with the combination of PBI-1737 and cyclophosphamide.

Methods and Results

In Vitro Studies

An *in vitro* migration assay was used to assess cell mobility in two dimensions. PC-3 cells were plated in a 12-well plate and grown to confluence in RPMI + 10% FBS. A rubber policeman was used to create a denuded area. Confluent cells were quiesced by mitomycin C treatment to prevent the confounding issue of cell proliferation. Cells were incubated in presence or absence of endothelial growth factor (EGF) and PBI-1737 for 24 h. Photographs were taken at 24 h.

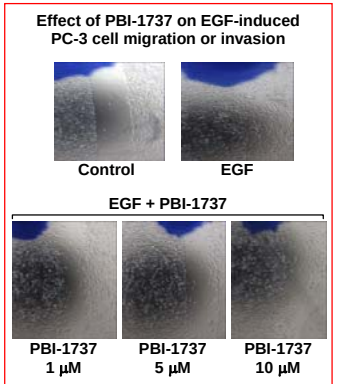

Figure 1

Figure 1 represents the effect of EGF and PBI-1737 on PC-3 cell migration. EGF promotes the migration of PC-3 cells treated with mitomycin compared to control (without growth factor). The addition of different concentrations of PBI-1737 to the cell culture induces an inhibition of the EGF-induced PC-3 migration.

In vitro cell-matrix adhesion was determined by fluorescence. Adherence of calcein-AM labeled PC-3 cells to laminin, collagen or matrigel was assessed using different doses of PBI-1737.

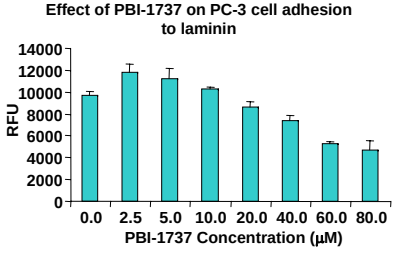

Figure 2

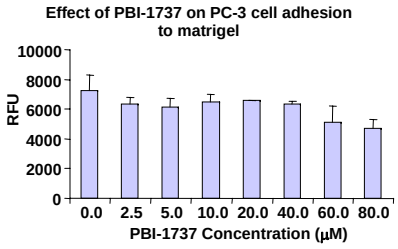

Figure 3

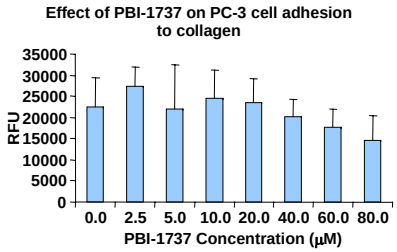

Figure 4

Figures 2, **3** and **4** represent the effect of PBI-1737 on PC-3 cell adhesion to laminin, matrigel and collagen, respectively. The addition of increasing concentrations of PBI-1737 inhibits in a dose dependent manner PC-3 cell adhesion to laminin, matrigel and collagen.

In Vivo Studies

The xenogenic human prostate tumor PC-3 cells were obtained from ATCC (CRL1435). Cells were grown in RPMI-1640 containing 10% fetal bovine serum. At day 0, 50 µl of viable PC-3 (1.5 to $2X10^6$) cells were injected intradermally to produce localized tumors in 6- to 8-week old male CD1 nu/nu mice (Charles River). Animals were monitored by manual palpation for evidence of tumor. When the tumors reached a satisfactory volume, mice were randomized and then treated with vehicle (saline), cyclophosphamide (Cytoxan; positive control, 100 mg/kg, iv once a week) or PBI-1737 (per os, 50 mg/kg, daily). Mice were sacrificed at day 57.

Figures 5 and **6** represent the antitumor efficacy of PBI-1737 with or without cyclophosphamide on the xenograft human prostate PC-3 tumor. Oral administration of PBI-1737 induces a significant ($p < 0.05$) inhibition of tumor volume with a T/C between 14% to 40%. Cyclophosphamide induces a significant inhibition ($p < 0.05$) of tumor volume with a T/C between 1% to 39%. Mice treated with the combination of cyclophosphamide and PBI-1737 demonstrated a significant ($p < 0.01$) inhibition of tumor volume with a T/C between 1% to 40% followed by tumor regression after day 43.

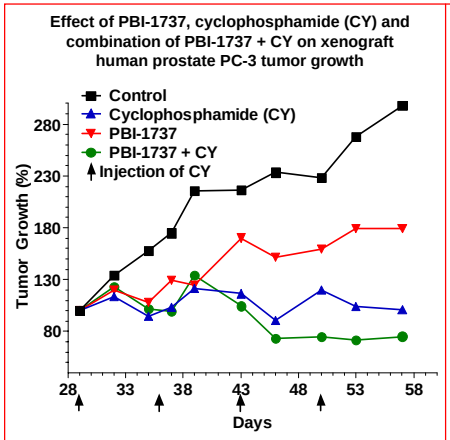

Figure 5

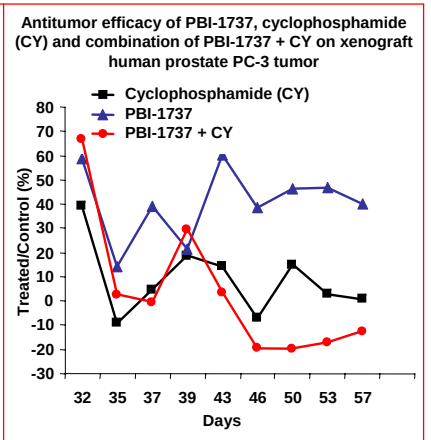

Figure 6

Conclusion

- PBI-1737 is a novel, low molecular weight, orally active molecule.
- PBI-1737 inhibits cell adhesion and migration of human prostate PC-3 cancer cells.
- PBI-1737 displays significant antitumor activity against human prostate cancer. A regression of the tumor is also observed with the combination of PBI-1737 and cyclophosphamide.

Taken together, these data suggest a promising role of PBI-1737 in the control of prostate tumor progression.