Supplemental Materials, Methods and Results for

Neutralizing Monoclonal Antibody to Periostin Inhibits Ovarian Tumor Growth and Metastasis

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There are 2 supplemental files for this manuscript:

1. Supplemental Materials, Methods (this Text)
2. Supplemental figures.
Supplemental Materials and Methods

PN immunohistochemistry. The immunostaining of PN was performed as described previously(1). Briefly, 4-micrometer frozen sections of tumor xenografts were fixed and stained with a specific rabbit anti-PN antibody. The detection employed universal biotinylated horse IgG in combination with ABC and substrate kits (Vector Laboratories, Burlingame, CA). Tissue sections were counterstained with hematoxylin, dehydrated, and mounted with Permount.

Western blot. PN western blots were performed as described previously(2). Briefly, the same amounts of tumor xenograft lysates were loaded onto a 6% SDS-polyacrylamide gel for electrophoresis, then proteins transferred to a nitrocellulose membrane and blotted with a PN-specific polyclonal antibody. After incubation with a horseradish peroxidase-conjugated anti-rabbit globulin (Transduction Laboratories, Lexington, KY), PN protein was visualized by a chemiluminescencet reagent (PerkinElmer Life Sciences, Boston, MA).

To analysis the activation of Akt, cells grown in SFM were treated with MZ-1 or control antibody at the final concentration of 20 μg/ml for 24 hrs. Cell lysates were prepared and blotted with anti-phospho-Akt antibody (Cell Signaling Technology Inc., Beverly, MA). Immunoblotting for β-actin was used as protein loading control.

2. Gillan L, Matei D, Fishman DA, Gerbin CS, Karlan BY, Chang DD. Periostin secreted by epithelial ovarian carcinoma is a ligand for alpha(V)beta(3) and alpha(V)beta(5) integrins and promotes cell motility. Cancer Res 2002;62:5358-64.