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Neuropilin-1, as a New Therapeutic Target in Human Pancreatic Cancer

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a deadly malignancy that is characterized by a high rate of mutations in the K-ras oncogene, the p53 tumor suppressor genes, and the p16 cell-cycle regulating gene, and by an abundance of transmembrane tyrosine kinase receptors and their ligands. These include the epidermal growth factor receptor, erb-B2, erb-B3, the insulin-like growth factor-1 receptor, and ligands such as transforming growth factor alpha, IGF-1, and vascular endothelial growth factor-A (VEGF-A).

Neuropilin-1 (Np-1), originally identified as a mediator of chemorepulsive factor for axons in the developing nervous system, is also expressed at high levels in the cancer cells in PDAC[1]. Np-1 is a transmembrane protein that acts as a co-receptor for VEGF-A and semaphorin 3A. The purpose of the present study was to assess the role of endogenous Np-1 in pancreatic cancer cells in relation to adhesion and invasion.

Methods

To evaluate the potential biological role of NP-1 in PDAC, we used PANC-1 human pancreatic cancer cells, which express high levels of endogenous Np-1. Parental PANC-1 cells were either sham-transfected, or transfected with the Np-1 antisense cDNA. To detect the association between Np-1 and integrin β1, we performed immunoprecipitation analysis followed by immunoblotting.

Using the adhesion and invasion assay, we analyzed the effect of down-regulation of Np-1 in relation to adhesion and invasion.

Results and Discussion

PANC-1 cells were transiently transfected with HA-tagged Np-1 cDNA. Immunoprecipitation of Np-1 with an anti-HA antibody followed by immunoblotting with an anti-integrin β1 antibody revealed a strong band corresponding to integrin β1 (**Fig. 1**), indicating that Np-1 forms a complex with integrin β1. Next, PANC-1 cells were transiently transfected with an HA-tagged cDNA encoding a truncated Np-1 that is devoid of the intracellular domain. Immunoprecipitation of a truncated Np-1 with an anti-HA antibody followed by immunoblotting with an anti-integrin β1 antibody again revealed a strong band corresponding to integrin β1 an extracellular domain of Np-1 (**Fig. 1**), indicating that Np-1 forms a complex with integrin β1. Immunoprecipitation of a truncated Np-1 with an anti-HA antibody followed by immunoblotting with an anti-integrin β1 antibody again revealed a strong band corresponding to integrin β1 (**Fig. 1**), indicating that integrin

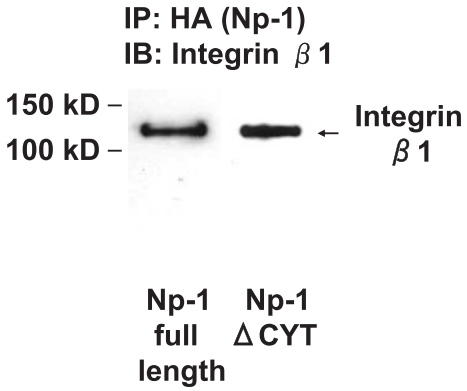

Fig. 1 Association of integrin β1 with Np-1

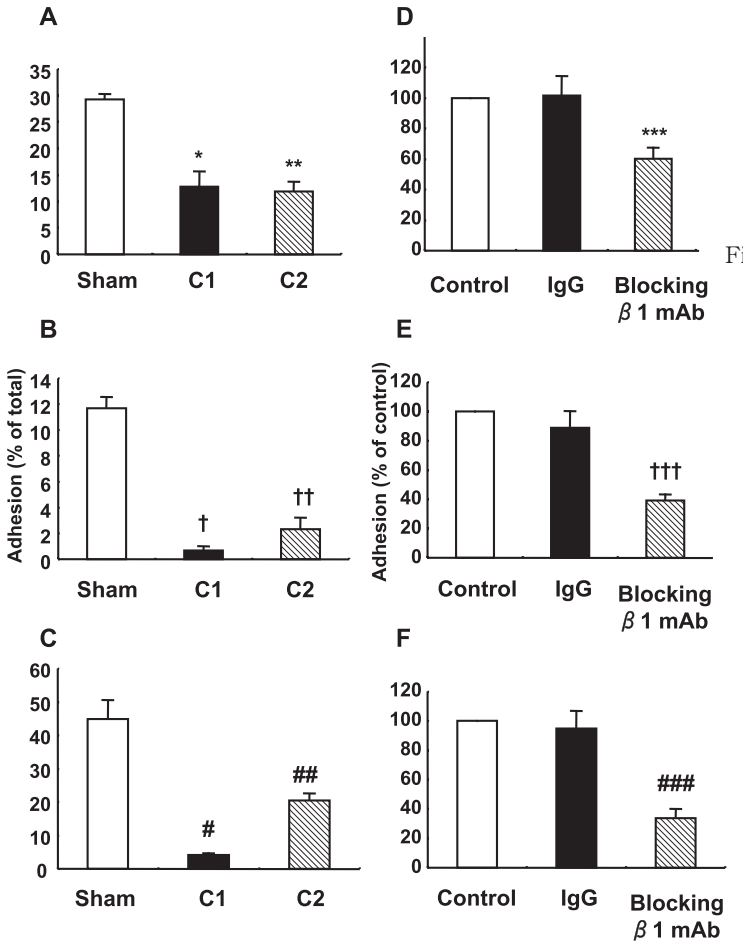

Fig. 2 Effects of anti-sense Np-1 and anti-integrin β1 antibodies on cell adhesion. **A – C**, Effects of Np-1 suppression. Sham transfected PANC-1 cells and clones 1 (C1) and 2 (C2) expressin the Np-1 antisense cDNA were seeded on plates coated with fibronectin (A), laminin (B), and collagen IV (C). *$p<0.01$, **$p<0.002$, †$p<0.001$, ††$p<0.002$, #$p<0.003$, and ##$p<0.02$ when compared with the corresponding values in sham transfected cells. **D – F**. Effects of anti-integrin β1 blocking antibody on cell adhesion. Cells were incubated in the absence or presence of 5 μg/mL anti-integrin β1 antibody or normal mouse IgG. Cells were then seeded in 96-well non tissue culture-treated plates coated with fibronectin (D), laminin (E), and collagen IV (F). ***$p<0.05$, †††$p<0.02$, and ###$p<0.02$ when compared with values observed with normal mouse IgG.

β1 associates with either the extracellular or transmembrane domains of Np-1[2].

By comparison with sham-transfected cells, both Np-1 antisense-expressing clones exhibited significantly decreased cell adhesion to fibronectin, laminin, and collagen IV **(Fig. 2)**. Similarly, by comparison with cells treated with a non-specific control IgG, cells treated with an antibody that disrupts integrin β1 function exhibited significantly decreased cell adhesion to fibronectin, laminin, and collagen IV **(Fig. 2)**[2].

Both Np-1 antisense-expressing clones exhibited a significantly decreased ability to invade across a matrigel membrane compared with sham-transfected cells **(Fig. 3)**. Moreover, VEGF did not enhance the invasiveness of sham-transfected cells, and did not restore the invasive capacity of Np-1 antisense-expressing clones **(Fig. 3)**[2].

In conclusion, Np-1 interacts with integrin β1 to coordinate signaling events that promote cell adherence and

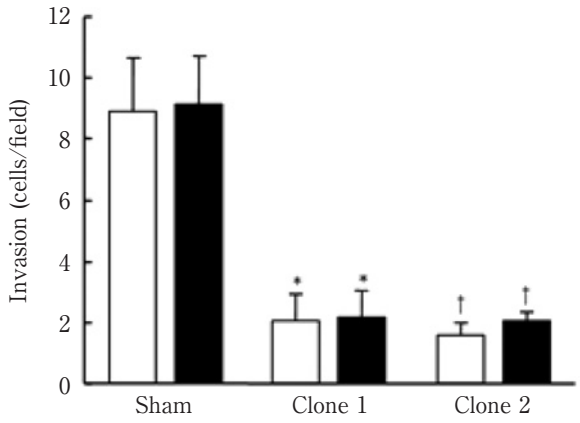

Fig. 3 Invasion assay. Sham-transfected and Np-1 anti-sense expressing clones were seeded on Matrigel in the upper cell culture inserts of a 24-well invasion chamber. The lower compartment was filled with the medium containing 0.5% FBS, in the absence (□) or presence (■) of 1 nM VEGF. *$p<0.03$ and †$p<0.02$ when compared with values for the corresponding sham transfected cells.

invasiveness. Therefore, targeting Np-1 in PDAC may enhance the effectiveness of other therapeutic modalities by suppressing cellular invasion.

References

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