Supplementary figure legends

Supplementary Figure 1. Expression of *Nectin-4* in normal tissues and lung tumors.

Twenty human lung-cancer cell lines and human bronchial epithelial cells (BEAS-2B) were grown in monolayers in appropriate media supplemented with 10% fetal calf serum (FCS) and were maintained at 37^{o}C in an atmosphere of humidified air with 5% CO_2. Semi-quantitative RT-PCR experiments were carried out as described elsewhere with synthesized *Nectin-4* gene-specific primers (5'-GTCAGCCAGAGGCTTGAACT-3' and 5'-GGATTCAAAGCAGGCACAGT-3'), or with β-actin (*ACTB*)-specific primers (5'-ATCAAGATCATTGCTCCTCCT-3' and 5'-CTGCGCAAGTTAGGTTTTGT-3') as an internal control (17, 18). ***Top and middle panels,*** Expression of *Nectin-4* in 14 NSCLCs (T; 7 clinical lung adenocarcinomas and 7 clinical lung squamous cell carcinomas) and corresponding normal lung tissues (N), examined by semi-quantitative RT-PCR. We prepared appropriate dilutions of each single-stranded cDNA prepared from mRNAs of clinical lung cancer samples, taking the level of β-actin (*ACTB*) expression as a quantitative control. ***Bottom panels,*** Expressions of *Nectin-4* in 20 lung cancer cell lines and normal bronchial epithelial cell, examined by semi-quantitative RT-PCR.

Supplementary Figure 2. Recognition of native Nectin-4 protein by monoclonal antibodies to Nectin-4 (clones 19-33 and 66-97).

Two monoclonal antibodies (clones 19-33 and 66-97) to Nectin-4 were proven to be specific for endogenous human Nectin-4, by immunocytochemistry, flow cytometry, and ELISA for the culture media using lung-cancer cell lines with high levels of endogenous Nectin-4 expression or those without Nectin-4 expression (**Fig. 1**), as well as by immunoprecipitation using COS-7 cells transfected with Nectin-4 expression vector. Exogenously expressed myc-tagged Nectin-4 protein in COS-7 cells was immunoprecipited by either of two monoclonal antibodies to Nectin-4 (clones 19-33 and 66-97) and immunoblotted with anti-myc antibodies. These monoclonal antibodies (19-33 and 66-97) could specifically immunoprecipitate Nectin-4 protein in myc-tagged Nectin-4-overexpressing cells (***left panel***), but not in mock-transformant cells (***right panel***), suggesting that these antibodies have an ability to specifically recognize the native Nectin-4.

Supplementary Figure 3. Growth promoting effect of Nectin-4.

A*, *In vitro enhanced growth of PC-14 cells stably expressing exogenous Nectin-4. To establish PC-14 cells stably expressing Nectin-4, Myc/His-tagged Nectin-4 expression vector (pcDNA3.1 myc/His-Nectin-4) or mock vector (pcDNA3.1-myc/His) was transfected into PC-14 cells that did not express endogenous Nectin-4 using FuGENE6 transfection reagent (Roche). Transfected cells were incubated in the culture medium containing 0.6 mg/mL neomycin (Geneticin, Invitrogen) for 14 days. Then, 50 colonies were trypsinized and screened for stable transfectants by a limiting-dilution assay. Expression of Nectin-4 was determined in each clone by RT-PCR, Western blotting and immunocytochemical staining. Cell viability of two stable clones (PC-14-Nectin-4-#A and -#B) and two control clones (PC14-Mock-#A and -#B) was quantified with MTT assay in 7 days. All assays were performed in triplicate wells three independent times. ***B*, *In vivo*** rapid growth of PC-14 cells stably expressing Nectin-4. The animal experiments were conducted according to the institutional and national guidelines for the care and use of laboratory animals, and approved by the institutional animal use committee. To examine *in vivo* tumor growth promotion by Nectin-4 overexpression, above established PC-14 cells stably expressing Nectin-4 or those transfected with mock plasmids (3×10^5 cells) were injected subcutaneously into the posterior dorsum of six BALB/cAJcl-*nu/nu* mice (female, 6 weeks old). Tumor volumes were measured and estimated for 20 days, by the following formula: $V = 0.5 \times (\text{longer diameter}) \times (\text{shorter diameter})^2$. ***Top panels,*** Expression of Nectin-4 protein in PC-14 cells injected subcutaneously into the posterior dorsum of BALB/cAJcl-*nu/nu* mice was detected by western blot analysis at 20 days after cell transplantation. ***Middle panels,*** Immunohistochemical evaluation of Nectin-4 expression in transplanted tumors at 20 days after cell transplantation (original magnification X 200). ***Bottom panel,*** Tumor growth curves in nude mice after inoculation of PC-14-Nectin-4#B cells (*red circle*; n = 3 mice) or PC-14-Mock-#B cells (*black square*; n = 3). Mean tumor volume ± 1SE was plotted.

Supplementary Figure 4. Inhibition of growth of NSCLC cells by siRNA against Nectin-4, and Enhancement of cellular invasiveness by Nectin-4 introduction into mammalian cells.

A, After 5-day incubation of NCI-H2170 and NCI-H358 cells (1×10^6 cells per dish) transfected with either of the siRNA oligonucleotides (100 nM), using 30 µl of Lipofectamine 2000 (Invitrogen), cell numbers were assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay using cell-counting kit-8 solution (DOJINDO LABORATORIES) (**Figs. 4*A* and 4*B***), and these cells were stained by Giemsa solution to assess colony formation. The image

of colony-formation assays of NCI-H2170 and NCI-H358 cells transfected with Nectin-4-specific siRNAs or control siRNAs was shown. ***B,*** Matrigel invasion assay was performed as described elsewhere using Matrigel matrix and invasion chambers (Becton Dickinson Labware), and COS-7 and NIH-3T3 cells transfected either with pcDNA3.1-myc/His plasmid designed to express human Nectin-4 or with mock plasmid (21, 25). ***Top panels,*** Transient expression of Nectin-4 in COS-7 and NIH-3T3 cells that were detected by western blot analysis. Immunoblotting was performed with anti-myc antibodies for myc/His-tagged Nectin-4 detection or anti-β-actin (ACTB) antibodies. ***Middle and bottom panels****,* Assays demonstrating the invasive nature of NIH-3T3 and COS-7 cells in Matrigel matrix after transfection with expression plasmids for human Nectin-4. Giemsa staining (***middle panels***; magnification, x100), and the relative number of cells migrating through the Matrigel-coated filters (***bottom panels***). Assays were done thrices and in triplicate wells.