Supplementary Methods

Small interfering RNA transfection

We used three Nampt-specific siRNAs and performed reverse transfections according to the instructions of the manufacturer. The sequences of the siRNAs were described in Supplementary Table S1. H358 and H1975 cells were incubated in 6-well plates in 0.5 ml Opti-MEM I reduced serum medium supplemented with 5 μl Lipofectamine RNAiMAX containing 20 nM Nampt-specific siRNA, 20 nM control siRNA or Alexa-Fluor 555-labeled RNA. Next, NSCLC cells were harvested and resuspended in 2.5 ml Dulbecco modified Eagle medium (DMEM) containing high glucose, 2 mM L-glutamine and 10% FBS, and 7.5–10.0 × 10^4 NSCLC cells were inoculated into each well and incubated for the indicated times. Western blotting and Alexa Fluor 555-labeled siRNA transfection were performed to evaluate the efficacy and efficiency of the siRNA- knockdown. H358 and H1975 cells were transfected with siRNAs for three days. H1975 cells transfected with Alexa Fluor 555-labeled siRNA were supplemented with Hoechst 33258 (Invitrogen, Carlsbad, CA, USA), and transfection efficiency was assessed by fluorescence microscopy (FV1000-D, Olympus, Tokyo, Japan). H358 and H1975 cells transfected with siRNAs were washed twice with

ice-cold PBS (pH 7.4) and lysed in Cell Lysis Buffer (CST, Tokyo, Japan). To determine the efficacy of Nampt-specific siRNAs, the expression of Nampt protein in the lysis solution was analyzed by Western blotting. Cell proliferation of lung cancer cells treated with Nampt-specific siRNA for the indicated times was evaluated by BrdU assays.

Cell proliferation assay

We used BrdU assays to assess cell proliferation. NSCLC cells were maintained in RPMI with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, 100 U/ml penicillin, and 100 mg/ml streptomycin (complete medium). The cells were harvested and 3.0–10.0 × 10^3 cells were incubated in 96-well plates. The next day, the medium was replaced by new complete medium with or without the indicated concentrations of gefitinib, and NSCLC cells were incubated for three days. After three days of gefitinib-treatment, a BrdU assay was performed according to the manufacturer's protocol. BrdU was added in the medium, and NSCLC cells were incubated for two hours. The BrdU-uptake in the treated cells was assessed using a microplate luminometer (Thermo Fisher Scientific, Waltham, MA, USA). For Nampt-specific siRNA transfection, H358 cells were transfected with siRNA for the indicated number of hours, and cell proliferation was determined by BrdU assay as described above.

Clonogenic growth assay

H1975 or LC2 cells (5.0–10.0 × 10^2 cells) were incubated in 6-well plates for 24 hours. Subsequently, growth medium was changed to new complete medium containing the indicated concentrations of FK866 or gefitinib. Treated cells were incubated under the normoxic condition for 14 days. After incubation, colonies in 6-well plate were fixed with 0.5% crystal violet (Wako, Japan) in 0.5% methanol. The number of colonies was determined by a colony counter and software (Microtec Nition, Funabashi, Japan).