**C-MYC inducible onco-lncRNA LINC00036 acting as EGFR mRNA stabilizer via RNA-protein and RNA-RNA interactions decreases the** **sensitivity of** **gefitinb in human cancer**

Shouping Xu1†, Lin Wan1†, Qin Wang1, Huizi Yin1, Kun Qiao1, Siwei Li1, Jinfeng Zhang1, Hao Wu1, Meiying Shen1, Shipeng Ning1 and Da Pang1,2\*

\* Correspondence: pangda@ems.hrbmu.edu.cn

† Shouping Xu and Lin Wan contributed equally to this article and should be regarded as joint first authors.

1Department of Breast Surgery, Harbin Medical University Cancer Hospital, 150 Haping Road, Harbin 150081, China

2 Heilongjiang Academy of Medical Sciences, 157 Baojian Road, Harbin 150086, China

**Supplementary information**

**Additional file 1:**

**Table S1**. Sequence of siRNAs and shRNAs used in this study.

**Table S2**. Antibody uesd in this study.

**Table S3**. Sequence of qRT-PR primers to detect RNA expression.

**Table S4**. Sequence of qRT-PR primers to detect microRNAs expression.

**Table S5**. Putative TF-binding site in the transcriptional start of LINC00036 locus.

**Table S6**. Predict microRNAs binding to the 3’-UTR of EGFR using public databases TargetScan and RNA22.

**Additional file 2:**

**Figure S1.** C-MYC mRNA expression is positively correlated with LINC00036 expression in human cancer. **A.** Knockdown of c-MYC decreases the expression of LINC00036 in GSE5823. **B**-**D.**c-MYC mRNA expression is positively correlated with LINC00036 expression in human cancer in LUSC (**B**), CESC (**C**) and ESCA (**D**) in TCGA data.

**Figure S2.** Knockdown LINC00036 inhibits cell growth and migration/invasion and accelerates cell apoptosis *in vitro*. **A.** Knockdown efficiency of LINC00036 in UACC-812, MDA-MB-453 cells via qRT-PCR analysis. **B.** CCK-8 assays for LINC00036 knockdown and Sh-NC group in UACC-812 cells and MDA-MB-453 cells. **C** and **D.** Representative images for flow cytometry(**C**) and quantification analysis (**D**) of analysis of UACC-812 cells and MDA-MB-453 cells after transfection**. E.** The [protein](javascript:;) [level](javascript:;) of Bax and Bcl-2 in LINC00036 knockdown group and Sh-NC group via western blot analysis. **F**-**I**. Cell migration and invasion and quantification analysis in MDA-MB-453and UACC-812 cells with LINC00036 knockdown. **J.** Knockdown efficiency of LINC00036 in NCI-H1975cells, SGC-7901 cells, SKOV-3 cells, 786-O cells, U251 cells, Hep-3B cells via qRT-PCR analysis.The data are presented as the mean± SD, \**P* < 0.05; \*\* *P* < 0.01; \*\*\* *P* < 0.001.

**Figure S3.** Characteristics of TTN and its toxicity effects on vital organs. **A.**The TUNEL assay for apoptotic cells in the TTN treatment group and in the other groups. **B.** H&E image for liver, kidney, heart, lung, spleen and skin treated with different treatments. (Original magnification. 200 ×. Scale bar: 200 µm). **C** and **D.**TUNEL assays for liver, kidney, heart, lung, spleen and skin treated with different treatments. (Original magnification. 200 ×. Scale bar: 100 µm). **E.** Serum biochemical indexes for detection of GPT, GGT, ALP, TBIL, CRE and BUN with different treatments. The data are presented as the mean± SD, \*\*\* *P* < 0.001.

**Figure S4.** LINC00036 expression is positively correlated with EGFR mRNA expression in human cancer. **A**-**I.** LINC00036 expression is positively correlated with EGFR mRNA expression in COAD (**A**), CESC (**B**), ESCA (**C**), HNSC (**D**), LUSD (**E**), PRAD (**F**), STAD (**G**), THCA (**H**) and THYM (**I**) in TCGA data. The data are presented as the mean± SD,\**P* < 0.05; \*\*\* *P* < 0.001.

**Figure S5.** LINC00036 promotes EGFR expression via RNA-protein interaction. **A.** LINC00036 sub-cellular localization assays with the nuclear and cytoplasmic fractions of UACC-812, MCF-7, 47D, NCI-H1950, NCI-H1650, SGC-7901, MKN-45, and MDA-MB-453 cells. The U1 small nuclear RNA was the nuclear positive control and GAPDH was the cytoplasmic positive control. **B.** RNA-FISH assays for LINC00036 sub-cellular localization in UACC-812, MDA-MB-452, SGC-7901 and NCI-H1975 cells. LINC00036 probes are shown in red. The U6 small nuclear RNA acted as the positive control (Original magnification: 1,000 ×; scale bar: 50 µm). **C.** Knockdown efficiency of si-PPP1R150 in T47D UACC-812 and SKBR3 cells via qRT-PCR analysis. **D.** Predicting the potential microRNAs that can bind to the 3′ UTR of *EGFR* using TargetScan and RNA22 databases. Seventy-four overlapping microRNAs were filtered between upregulated microRNAs (FC > 1.5, *P* < 0.05) in the BGISEQ-500 RNA-seq of the HMUCC cohort and the above predicted microRNAs in databases. Venn diagrams were drawn using the Venny online software analysis. **E** and **F.** Expression of LINC00036 in T47D and UACC-812 cells treated with microRNA inhibitors of miR-424-5p, miR-196b-5p, miR-301a-5p, miR-708-5p, miR-125b-2-3p, miR-143-3p, miR-503-5p, miR-324-3p, miR-1260a, miR-1260b, miR-4510, and miR-6720-5p. **G** and **H.** Expression of EGFR mRNA in T47D and UACC-812 cells treated with microRNA inhibitors or mimics of miR-424-5p, miR-196b-5p, miR-301a-5p, miR-708-5p, miR-125b-2-3p, miR-143-3p, miR-503-5p, miR-324-3p, miR-1260a, miR-1260b, miR-4510, and miR-6720-5p. The data are presented as the mean ± SD, \**P* < 0.05; \*\* *P* < 0.01; \*\*\* *P* < 0.001; \*\*\*\* *P* < 0.0001.

**Figure S6.** LINC00036-miR-125b-2-3p/miR-424-5p-EGFR axis promotes cell proliferation. **A** and **B.** Cell proliferation was analyzed by CCK-8 assay in NCI-H1975 and SGC-7901cell. The data are presented as the mean± SD, \*\* *P* < 0.01; \*\*\* *P* < 0.001; #*P* < 0.05. \*\* *P* < 0.01; \*\*\* *P* < 0.001 vs Sh-NC; #*P* < 0.05 vs Sh-LINC00036-1.

**Figure S7.** Downregulation of LINC00036 increases the sensitivity of cancer cells to gefitinib. **A**-**H.** CCK8 assays were measure the IC50 ability of LINC00036 knockdown in MDA-MB-231, UACC-812, NCI-H1975, A549, SGC-7901, 786-O, Hep3B and SK-OV-3 cells after various concentration of gefitinib treatment for 48 h. **I.** Cell apoptosis in combination of LINC00036 knockdown and gefitinib reatment group, LINC00036 knockdown group and gefitinib treatment alone group in UACC-812 tumor mice models by TUNEL assays.The data are presented as the mean± SD, \**P* < 0.05; \*\*\* *P* < 0.001