# Interleukin-17 promotes angiogenesis by stimulating VEGF production of cancer cells via the STAT3/GIV signaling pathway in non-small-cell lung cancer

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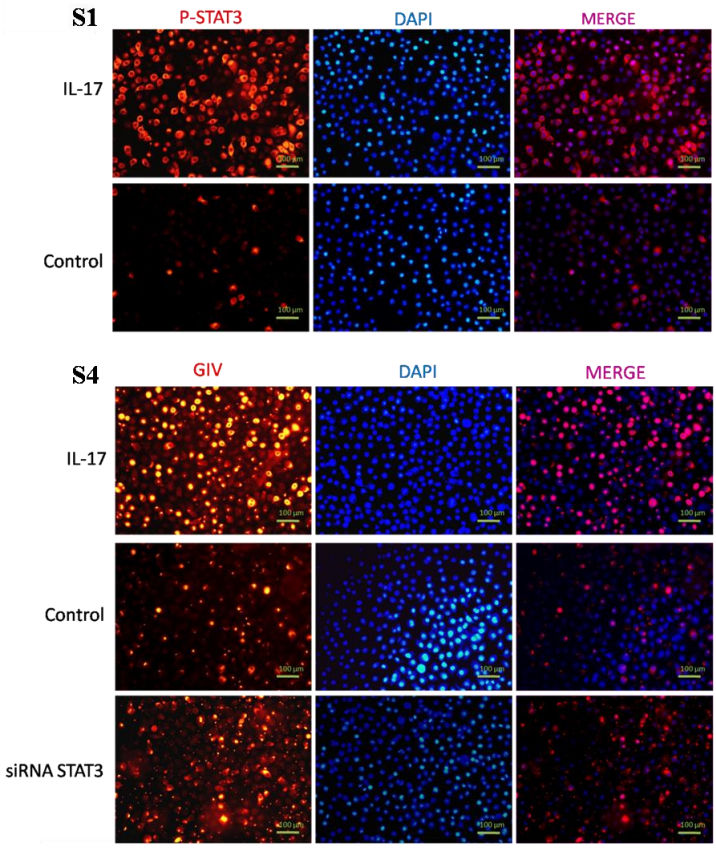





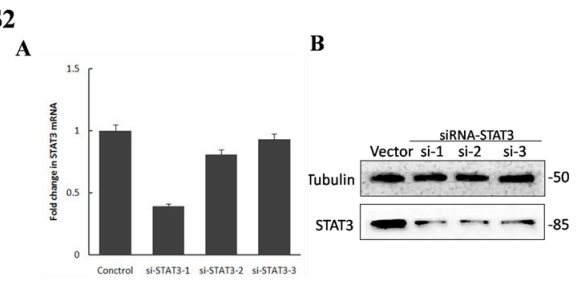


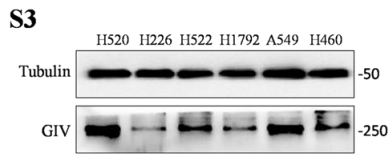


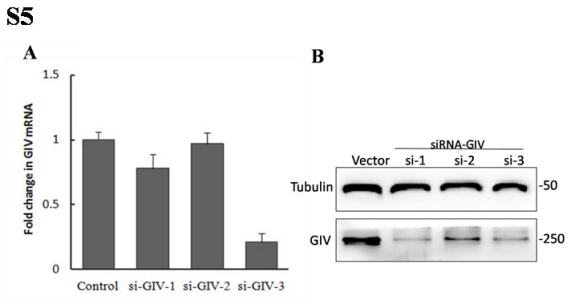


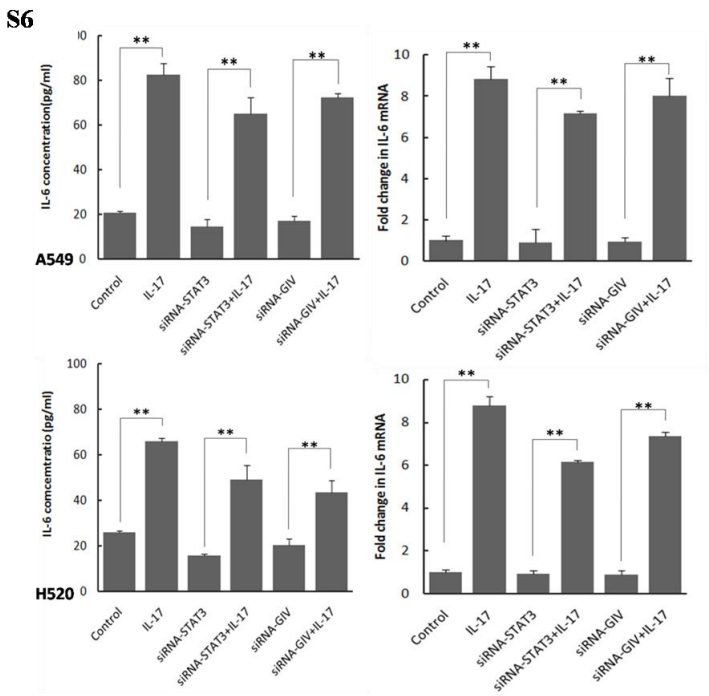


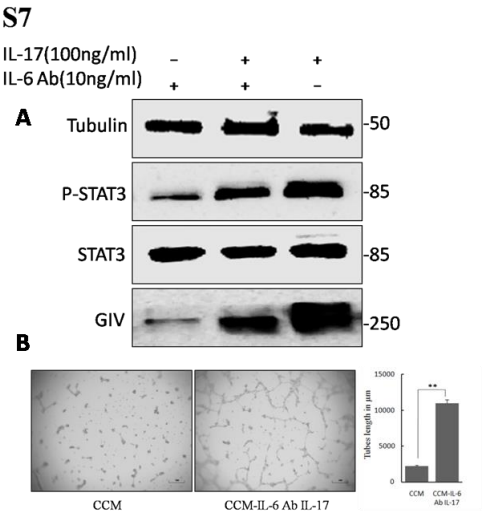

## Figures legends

**Fig.S1** Immunofluorescence assays showed that recombinant human IL-17(100ng/ml for 24h) significantly elevated the expression of p-STAT3 in H520 cells. Photomicrographs were taken at ×200 magnification. Control, PBS.

**Fig.S2** A549 cells were transfected with control or STAT3 siRNAs. The cells were incubated for 48 h, and the cell lysates were analyzed by (A) real-time PCR and (B) Western blot analyses.

**Fig.S3** Western blotting showed that expression of GIV in NSCLC cell lines.

**Fig.S4** Immunofluorescence assays showed that recombinant human IL-17(100ng/ml for 24h) significantly elevated the expression of GIV in H520 cells. However, the expression of GIV decreased if depleted of endogenous STAT3. The photomicrographs were taken at ×200 magnification.

**Fig.S5** A549 cells were transfected with control or GIV siRNAs. The cells were incubated for 48 h, and the cell lysates were analyzed by (A) real-time PCR and (B) Western blot analyses.

**Fig.S6** NSCLC cells were cultured for 24h with IL-17(100ng/ml) or PBS. Concentrations of IL-6 in culture supernatants were measured by ELISA, and mRNA of IL-6 in NSCLC cells were measured by Real-time PCR. IL-17 up-regulated the production of IL-6 by tumor cells, while both in siRNA-STAT3 and siRNA-GIV cells, IL-17-induce d IL-6 upregulation was not affected.

**Fig.S7** IL-17 partially activates STAT3 and increased tube formation via IL-6 induction. A549 cells were treated with control, IL-17 or IL-17 and IL-6 mAb (5ng/ml). Cell lysates were analyzed by western blot(A) and CCM were harvested added to HUVECs plated on Matrigel for tube formation assay(B).

**Table S1** IL-17 affects the production of proangiogenic factors of NSCLC

| | H1792(pg/ml) | | | H460(pg/ml) | | |
|---|---|---|---|---|---|---|
| Angiogenic factors | Control | IL-17 | p-value | Control | IL-17 | p-value |
| IL-6 | 47.7±4.5 | 83.4±10.2 | 0.040 | 43.9±0.4 | 103.0±1.8 | ＜0.001 |
| IL-10 | 28.8±0.7 | 28.9±0.8 | 0.421 | 23.8±2.0 | 24.2±1.4 | 0.102 |
| IL-8 | 3353.5±14.0 | 2789.1±22.8 | 0.126 | 110.3±10.2 | 388.9±30.9 | 0.050 |
| bFGF | 65.2±2.6 | 84.3±0.5 | 0.100 | 87.8±2.3 | 84.3±3.7 | 0.054 |
| VEGF | 570.5±17.6 | 1069.9±5.7 | 0.012 | 493.9±1.1 | 1772.1±19.5 | 0.043 |
| PDGF | 15.5±0.4 | 12.3±0.3 | 0.293 | 15.0±1.6 | 20.6±2.0 | 0.658 |
| Endostain | 41.8±0.1 | 31.3±0.3 | 0.114 | 36.4±0.1 | 21.5±0.1 | 0.463 |

ELISA-determined cytokine levels were exprssed as means±SD. *p*-values were calculated by student's *t* test.

**Table S2** IL-17 and GIV expression in NSCLC and the relationship with patients' characteristics.

| | | IL-17 | | | GIV | | |
|---|---|---|---|---|---|---|---|
| Pathological features | Total | Low | High | *p*-value | Low | High | *p*-value |
| Age(years) | | | | | | | |
| ＜65 | 36 | 19 | 17 | | 21 | 15 | |
| ≥65 | 31 | 12 | 19 | 0.250 [a] | 12 | 19 | 0.109 [a] |
| Gender | | | | | | | |
| Female | 27 | 12 | 15 | | 12 | 15 | |
| Male | 40 | 19 | 21 | 0.806 [a] | 21 | 19 | 0.518 [a] |
| Smoking status | | | | | | | |
| Smoker | 49 | 23 | 26 | | 24 | 25 | |
| Non-smoker | 18 | 8 | 10 | 0.856 [a] | 9 | 9 | 0.941 [a] |
| Tumour stage | | | | | | | |
| I and II | 35 | 21 | 14 | | 19 | 16 | |
| III and IV | 32 | 10 | 22 | 0.018 [a] | 14 | 18 | 0.389 [a] |
| Histological type | | | | | | | |
| Squamous cell carcinoma | 26 | 10 | 16 | | 12 | 14 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Adenocarcinoma | 33 | 17 | 16 | | 18 | 15 | |
| Others | 8 | 4 | 4 | 0.580[b] | 3 | 5 | 0.657[b] |
| Differentiation | | | | | | | |
| Well differentiated | 24 | 12 | 12 | | 10 | 14 | |
| Moderately-poorly | 43 | 19 | 24 | 0.647[a] | 23 | 20 | 0.353[a] |

[a] Pearson's $\chi^2$ test.

[b] Fisher's exact test

**Table S3** Univariate and multivariate analyses of factors associated with survival

| | A | | B | | C | |
|---|---|---|---|---|---|---|
| Variables | HR (95% CI) | *p* | HR (95% CI) | *p* | HR (95% CI) | *p* |
| Gender(female vs. male) | NA | NA | NA | NA | NA | NA |
| Age, years(＜65 vs. ≥65) | NA | NA | NA | NA | NA | NA |
| Smoking status(smoker vs. nonsmoker) | NA | NA | NA | NA | NA | NA |
| TNM stage (I-II vs. III-IV) | 0.311(0.165-0.586) | ＜0.001 | 0.293(0.154-0.557) | ＜0.001 | 0.294(0.152-0.568) | ＜0.001 |
| Differentiation (well vs. Moderate-poor) | NA | NA | NA | NA | NA | NA |
| Histological type (ADC vs. non-ADC) | NA | NA | NA | NA | NA | NA |
| IL-17 expression (low vs. high) | NA | NS | NA | NA | NA | NA |
| GIV expression (low vs. high) | NA | NA | 0.467(0.250-0.872) | 0.016 | NA | NA |
| Combination of IL-17 and GIV* | | | | | | |
| Overall | NA | NA | NA | NA | NA | 0.004 |
| I vs II | NA | NA | NA | NA | 3.141(1.484-6.647) | 0.002 |
| I vs III | NA | NA | NA | NA | 2.544(1.154-5.606) | 0.017 |

Note: Multivariate analysis was performed using the Cox multivariate proportional hazard regression model with a stepwise method (forward, likelihood ratio).

Abbreviations: HR, hazard ratio; CI, confidence interval; ADC, adenocarcinom; NA, not assessed; NS, not significant; TNM, tumor-node-metastasis.

*I, IL-17 high and GIV high expression; II, IL-17 high, GIV low expression or IL-17 low, GIV high expression; III, IL-17 low and GIV low expression.