

Background

Stimulator of interferon genes (STING) is a central mediator of the cytosolic DNA-sensing pathway that induces type I interferon and inflammatory signaling. Although cGAS-STING signaling has been investigated in the context of EGFR-TKI resistance in EGFR-mutant lung cancer,^{1,2)} the precise role of STING signaling in the progression of these tumors and its relationship with EGFR signaling remain unclear. Although high STING expression generally predicts favorable outcomes in unselected lung cancer patients, we previously found that it is associated with poorer recurrence-free survival in surgically resected EGFR-mutant lung cancer compared to STING-low tumors (5-year RFS: 55.9% vs. 92.9%, $p = 0.0382$). Furthermore, STING-high tumors exhibited elevated EGFR copy numbers, suggesting a link to chromosomal instability.³⁾ Therefore, we investigated whether STING activation contributes to EGFR signaling and malignant potential using EGFR-mutant lung cancer cell lines.

Materials and Methods

Cell lines and reagents

The EGFR-mutant cell lines used in this study included PC9 and H1650 (exon 19 deletion), as well as H1975 (L858R/T790M). H1650 is additionally PTEN-deficient. The A549 cell line, which harbors a KRAS G12S mutation, was utilized as an EGFR wild-type control. 2',3'-cGAMP, H-151, and G150 were purchased from Selleck Chemicals, and cell lysis buffer and antibodies used for western blotting were purchased from Cell Signaling Technology.

Western blotting

Cells were treated with the indicated drugs or an equal volume of DMSO, and lysed in cell lysis buffer. Equal amounts of protein from the cell lysates were separated by SDS-PAGE and then transferred to nitrocellulose membranes. After blocking, the membranes were incubated with primary antibodies overnight at 4 °C. The primary antibodies were detected using horseradish peroxidase-conjugated secondary antibodies and visualized with an enhanced chemiluminescent detection system (Amersham Biosciences).

Immunofluorescence

Cells were seeded in 4-well chamber slides at 3,000 cells/well and treated with the indicated drugs. Cells were fixed with 4% paraformaldehyde, permeabilized with 0.2% Triton X-100, blocked with 5% BSA, and stained with an anti-STING antibody (CST, #90947; 1:3200) and an anti-EGFR antibody (Invitrogen, MA1-12693; 1:200). Alexa Fluor 488-conjugated goat anti-rabbit IgG (Abcam, #ab150077) and Alexa Fluor 568-conjugated goat anti-mouse IgG (Invitrogen, #A-11004) were used as secondary antibodies. Images were acquired using an LSM 900 confocal microscope (Carl Zeiss) with a 100 × oil objective.

Quantitative RT-PCR (RT-qPCR)

Total RNA was extracted using Sepasol-RNA I Super G (Nacalai Tesque), reverse-transcribed using ReverTra Ace qPCR RT Master Mix (TOYOBO), and analyzed using SYBR qPCR Mix (TOYOBO) on a LightCycler 96 system. ACTB was used as the reference gene, and relative expression was calculated by the $2^{-\Delta\Delta C_q}$ method and shown as a log2 fold change.

Discussion / Conclusion

In this study, cGAMP-induced STING activation was associated with cell line-dependent changes in EGFR downstream phosphorylation, particularly in EGFR-mutant lung cancer cell lines. Immunofluorescence analysis further showed that cGAMP increased the apparent spatial overlap between STING and EGFR signals, which is consistent with recent reports linking mutant EGFR to STING–TBK1 signalosomes.⁴⁾ In contrast, EGFR/ligand-related transcripts tended to decrease after cGAMP stimulation, suggesting that EGFR signaling changes may not be explained by a simple ligand-mediated autocrine loop. H-151 and G150 showed limited and heterogeneous effects on EGFR downstream signaling, indicating that STING/EGFR-associated signaling may be regulated in a cell line- and treatment-dependent manner. Together with our previous clinical findings, these results suggest that STING may be involved in EGFR-associated malignant signaling in EGFR-mutant lung cancer.

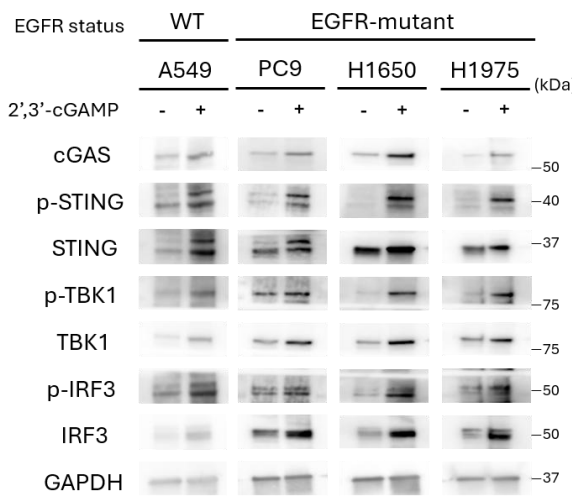
References

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- Yonesaka K, et al. Cells. 2025;14(6):447.
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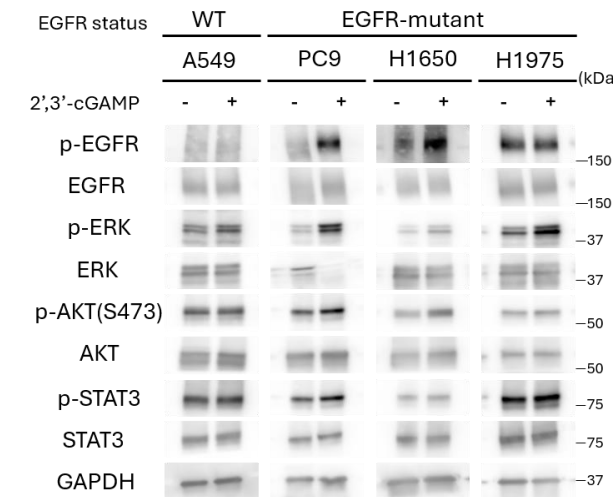
Results

Figure 1. Acute cGAMP activates STING signaling and differentially modulates EGFR downstream signaling (2',3'-cGAMP, 10 μ M, 6 h)

A. STING signaling



B. EGFR-downstream signaling

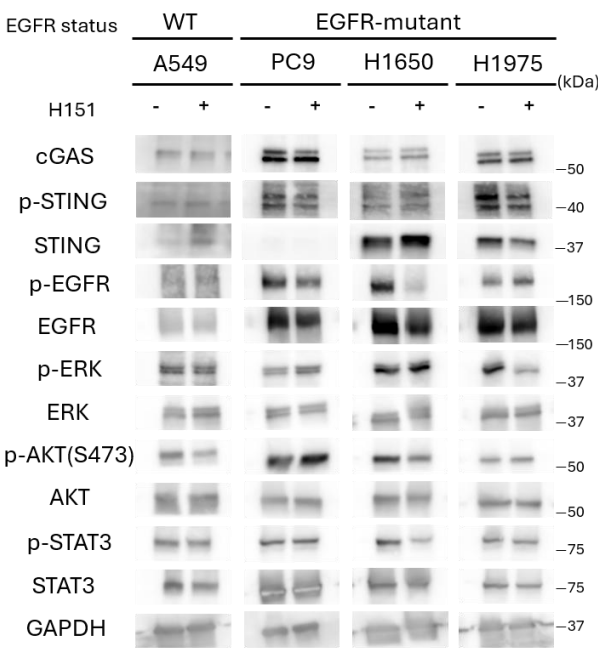


- Acute cGAMP stimulation activated the STING–TBK1–IRF3 signaling axis.
- EGFR downstream signaling changes appeared more evident in EGFR-mutant cell lines than in EGFR-wild-type A549.
- These findings suggest a potential crosstalk between STING activation and EGFR-associated signaling in EGFR-mutant lung cancer cells.

Figure 2. Effects of STING/cGAS inhibition on EGFR downstream signaling

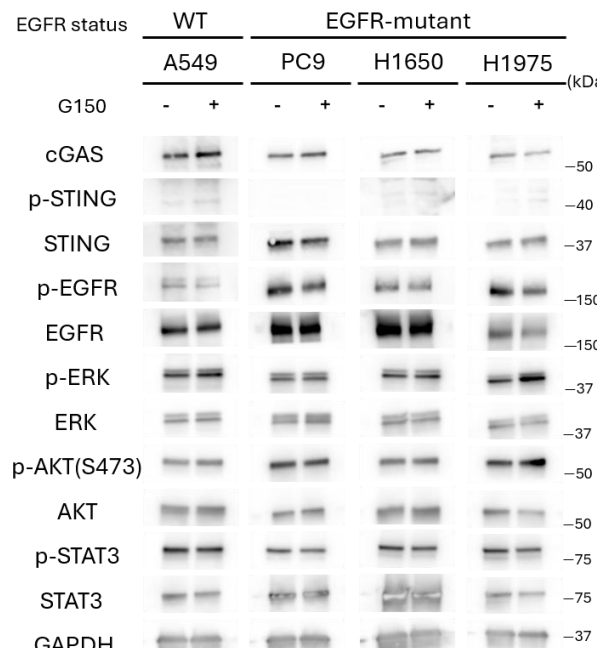
A. H-151 treatment

STING inhibitor, 10 μ M, 6 h



B. G150 treatment

cGAS inhibitor, 10 μ M, 6 h



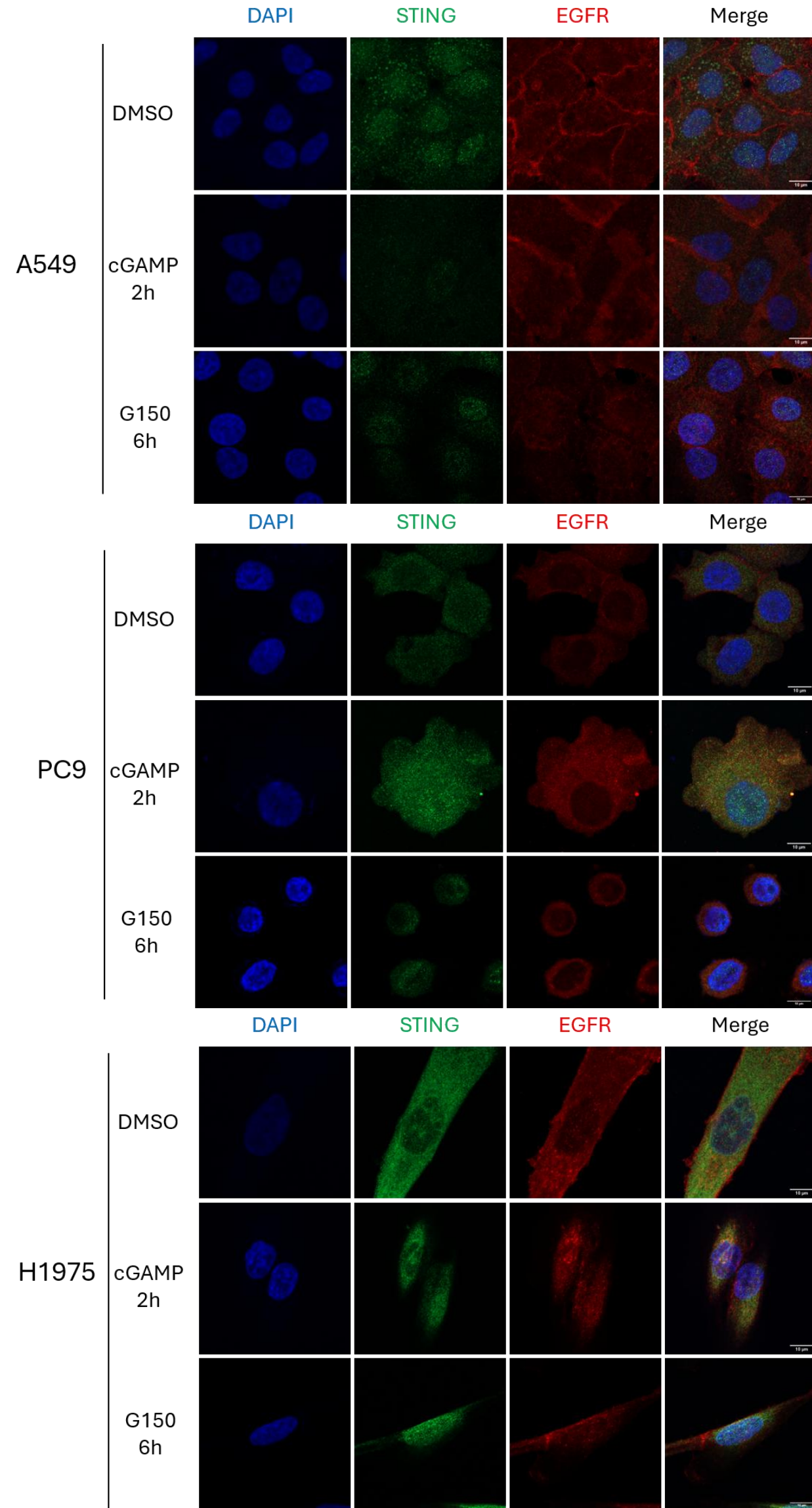
- Unlike cGAMP stimulation, H-151 and G150 did not induce obvious activation of EGFR downstream signaling.
- Some phosphorylation signals appeared reduced in selected cell lines, but the effects were cell line-dependent.

Gene	A549	PC9	H1650	H1975
STING	-0.30	-1.87	0.42	-0.73
IFNB1	0.55	0.89	1.69	2.97
CXCL10	4.94	0.24	1.84	2.44
EGFR	-0.49	-0.67	-1.40	-0.97
EGF	-1.32	-0.90	0.07	-0.47
HBEGF	-1.27	-2.87	-1.09	-0.86
AREG	-0.75	-2.25	-0.91	-2.15
EREG	-0.82	-2.85	-0.48	-1.68
IL6	-0.83	-0.59	-0.99	-0.50
VEGFA	-1.89	-1.11	-1.80	-1.58
CDH1	-0.99	-0.68	-1.30	-0.50
VIM	-0.46	-0.82	-1.76	-0.90
SNAI1	0.34	-0.05	-1.71	-0.13

Figure 3. qPCR heatmap of cGAMP-induced transcriptional changes 2',3'-cGAMP, 10 μ M, 6 h vs DMSO; log2 fold change

- cGAMP induced cell line-dependent transcriptional remodeling.
- IFN-associated genes were induced in selected cell lines, whereas EGFR/ligand-related genes tended to decrease.
- These findings suggest that EGFR signaling modulation may not be explained by a simple ligand-mediated autocrine loop.

Figure 4. STING/EGFR localization after cGAMP or G150 treatment (2',3'-cGAMP, 10 μ M, 2 h; G150, 10 μ M, 6 h)



Scale bar, 10 μ m.

- cGAMP stimulation increased the apparent spatial overlap between STING and EGFR signals, particularly in EGFR-mutant cell lines.
- In contrast, G150-treated cells showed distinct STING and EGFR localization patterns with less apparent overlap.
- These findings suggest that cGAMP-induced STING activation may alter the spatial relationship between STING and EGFR.