The impact of targeting TRAF2 and NCK-interacting protein kinase (TNIK) on anti-tumor effect in small cell lung cancer

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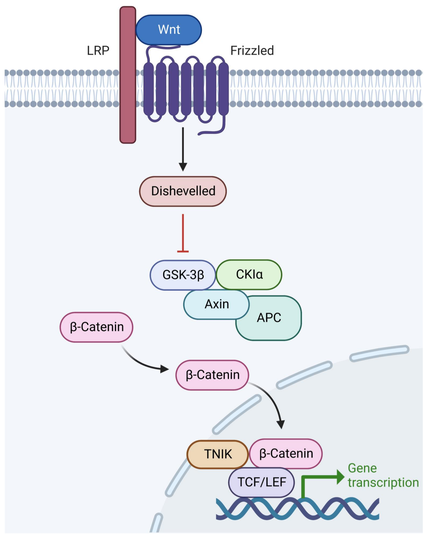
Background

Small cell lung cancer (SCLC) is a highly lethal malignancy, with rapidly acquired chemotherapy resistance. Some studies have reported that Wnt signaling pathway activation promoted cell proliferation and was correlated with chemo-resistance in SCLC (1-3). Additionally, our group has demonstrated that cisplatin relapsed model generated high mesenchymal subgroup using single-cell analysis (4). None of the therapies targeting Wnt pathway components of the transmembrane and the cytoplasm have been successful in a clinical application due to toxicity and insufficient efficacy (5). However, targeting Wnt signaling inside the nucleus has been drawing increasing attention as cancer therapeutics. TRAF2 and NCK-interacting protein kinase (TNIK), which interacts with downstream effectors, TCF4/β-catenin transcriptional complex, is an essential activator of Wnt target genes (6). TINIK is highly expressed in several cancers for cell proliferation, thus TNIK is expected as a novel druggable target (7-11). On the other hand, the question remains whether TNIK is a critical target in SCLC.

Outline of the role of TNIK in Wnt/β-catenin pathway

Wnt ligand binds to its receptors (Frizzled and LRP5/6), followed by dishevelled (Dsh) protein becomes activated. Dsh inhibits GSK-3β/Axin/APC complex and subsequently dephosphorylates β-catenin. Accumulated β-catenin in cytoplasm moves to the nucleus and binds to TCF4. TNIK is required for activation of the TCF4/β-catenin complex and initiates transcription of Wnt target genes.



Hypothesis

We hypothesize that a TNIK inhibitor has potent anti-tumor effects in SCLC and its promising biomarkers exist.

Experimental Design

- We evaluated susceptibility to a TNIK inhibitor, NCB-0846 in 29 human-derived SCLC cell lines using 96-hour proliferation assays.
- We correlated NCB-0846 IC50 values with proteomic profiling (Reverse Phase Protein Array, RPPA) data.
- We reduced TNIK expression using siRNA against TNIK in human SCLC cell lines.
- We assayed DNA damage protein changed by NCB-0846 using western blot in human SCLC cell lines.

COI : I have no financial relationships to disclose.

Finding 1: A TNIK inhibitor significantly reduces cell viability of SCLC-N and SCLC-P subtype.

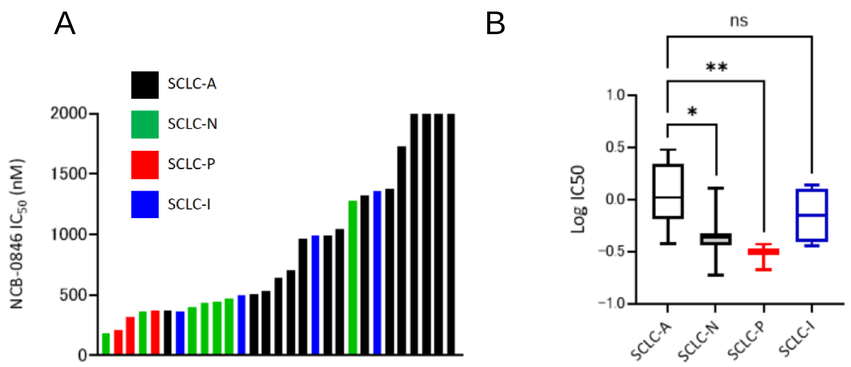


Fig 1. A novel TNIK inhibitor, NCB-0846 showed potent activity in SCLC-N and SCLC-P cell lines. (A) IC50 values of NCB-0846 after 96h treatment in all subtypes of SCLC cell lines. **(B)** Boxplots for IC50 values of NCB-0846 by subtype. *, P<0.05, **, P<0.01

Finding 2: High levels of cMYC and TTF1 were strongly correlated with in vitro sensitivity and resistance to the TNIK inhibitor.

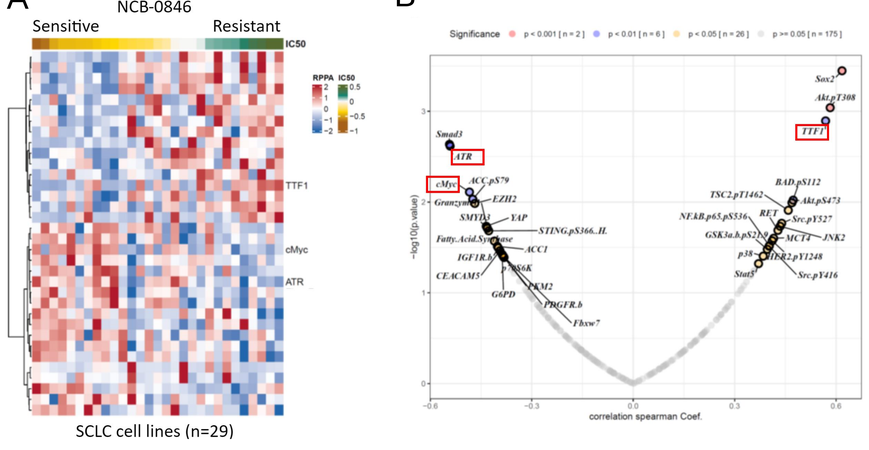


Fig 2. cMYC, ATR, and TTF1 are predictive biomarkers response to the TNIK inihibitor in SCLC. (A) RPPA heatmap showing expression changes that correlate with IC50 values in human SCLC cell lines. **(B)** Volcano plot of Spearman coefficients (x-axis) versus p-values (y-axis). Labels were added for proteins where p-values for association were below 0.05.

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Finding 3: TNIK inhibition reinforces sensitivity to cisplatin in cisplatin-resistant SCLC cells.

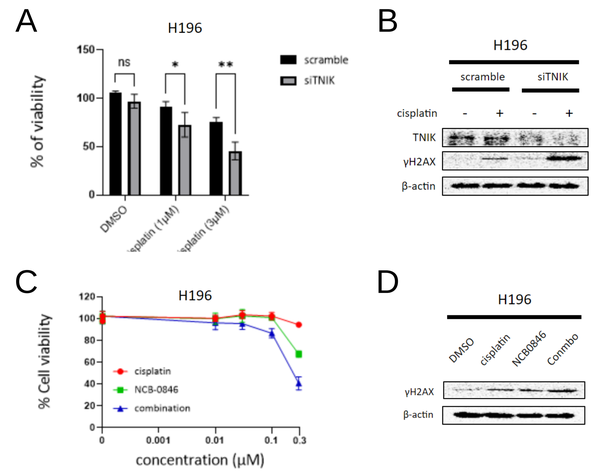
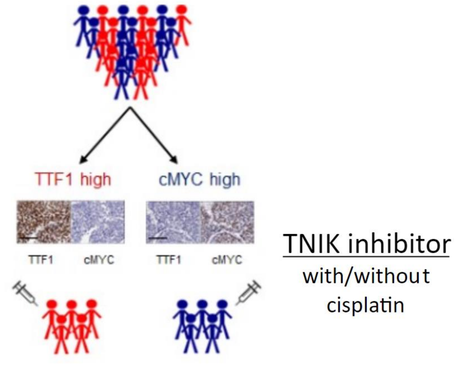


Fig 3. TNIK inhibition reduces resistance to cisplatin in SCLC cells. (A) Cell viability in cisplatin-resistant SCLC cells treated with either scrambled siRNA or siRNA targeting TNIK. The cells were treated with DMSO, cisplatin (1 μmol/L) and cisplatin (3 μmol/L) for 96 h. *, P<0.05, **, P<0.01 **(B)** Western blotting of the SCLC cells with TNIK which were treated with cisplatin (1 μmol/L) for 48h. **(C)** Cell viability determined after treated with escalating doses of cisplatin, NCB-0846, and combination of them for 96 h. **(D)** Western blotting of the cells treated with DMSO, cisplatin (1 μmol/L) and/or NCB-0846 (500 nmol/L) for 48 h.

Summary and Future Directions

- Our preclinical results indicate that cMyc and TTF-1, which negatively correlated (12), are positive and negative predictive marker of the efficacy of NCB-0846 in SCLC cell lines.
- Findings support TNIK inhibitors are selected for patients with SCLC expressing cMYC high/TTF1 low, which is immunochemically distinguishable (Fig. 4).
- Further studies should test combination of NCB-0846 and cisplatin in cMyc-high/TTF1-low SCLC *in vivo* models.

Fig 4. Working model of how a TNIK inhibitor may be selected based on TTF1 and cMYC IHC. Example TTF1 and cMYC IHC from two archived SCLC tumor samples on a neuroendocrine TMA (scale bar = 100μm).



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