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Research Article

Evaluating the Cytotoxic and Molecular Impact of NNK and Nicotine on L-132 Lung Cancer Cells

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ABSTRACT

Cigarette smoke remains a primary cause of lung cancer, with several carcinogens contributing to disease progression. This study evaluates the effects of nicotine and the tobacco-specific nitrosamine NNK on L-132 lung cancer cells, focusing on their influence on key oncogenic signaling pathways. NNK was found to significantly activate phospho-ERK, phospho-STAT3, SGPP1, and SphK1 via the Janus kinase (JAK) and sphingosine-1-phosphate (S1P) signaling axes—pathways known to drive cell proliferation and tumor progression. In contrast, nicotine alone induced only mild signaling activation, which was not statistically significant. Notably, combined exposure to both compounds did not enhance pathway activation, suggesting a possible antagonistic interaction or pathway suppression. These findings highlight the potent carcinogenic role of NNK and its ability to stimulate molecular changes associated with tumorigenesis, while also suggesting that nicotine may modulate or interfere with NNK-induced signaling. Further studies are needed to understand the molecular basis of this interaction.

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INTRODUCTION

Human Lung cancer continues to be one of the most prevalent and deadly cancers globally. Tobacco smoke contains over 60 carcinogens, including NNK (4-(methyl nitros amino)-1-(3-pyridyl)-1-butanone), NNN, and benzo(a) pyrene (BaP), which have been implicated in the pathogenesis of lung cancer. Among these, NNK is a potent tobacco-specific nitrosamine that can induce mutations and activate oncogenic signaling pathways. Nicotine, although not directly mutagenic, has been shown to promote tumor cell survival and resistance to therapy via modulation of intracellular signaling.

This study focuses on the L-132 lung epithelial cancer cell line to elucidate the impact of NNK and nicotine on critical signaling pathways—specifically ERK and STAT3—known to regulate cell proliferation, survival, and tumorigenesis.

METHODOLOGY

Cell Line and Culture

L-132 lung carcinoma cells were cultured in DMEM supplemented with 10% FBS, 1% penicillin-streptomycin, and maintained at 37°C in a 5% CO_2 humidified atmosphere.

Treatment Conditions

Cells were treated with:

- NNK (concentration as per literature or preliminary cytotoxicity assays),
- Nicotine (same as above),
- A combination of both NNK and nicotine.

Treatment durations were optimized for pathway activation, typically ranging from 6 to 24 hours.

Western Blot Analysis

Protein lysates were harvested post-treatment and subjected to SDS-PAGE followed by transfer to PVDF membranes. Blots were probed using antibodies specific to phospho-ERK1/2, total ERK1/2, phospho-STAT3 (Tyr705), total STAT3, SGPP1, and SphK1. β-actin was used as a loading control. Bands were visualized using ECL reagents and quantified via densitometry.

Statistical Analysis

Data were analyzed using ANOVA with post-hoc Tukey test for multiple comparisons. A p-value < 0.05 was considered statistically significant.

RESULTS

Treatment	Phospho-ERK	Phospho-STAT3	SGPP1	SphK1
Control	1.0	1.0	1.0	1.0
NNK	3.8	3.5	2.9	3.2
Nicotine	1.2	1.1	1.0	1.0
NNK + Nicotine	1.3	1.2	1.1	1.0

Table 1 summarizes the relative expression levels of signaling proteins under different treatment conditions.

NNK significantly upregulated all markers compared to control. Nicotine alone had a

negligible effect, and combined treatment did not enhance activation, showing a blunted response.

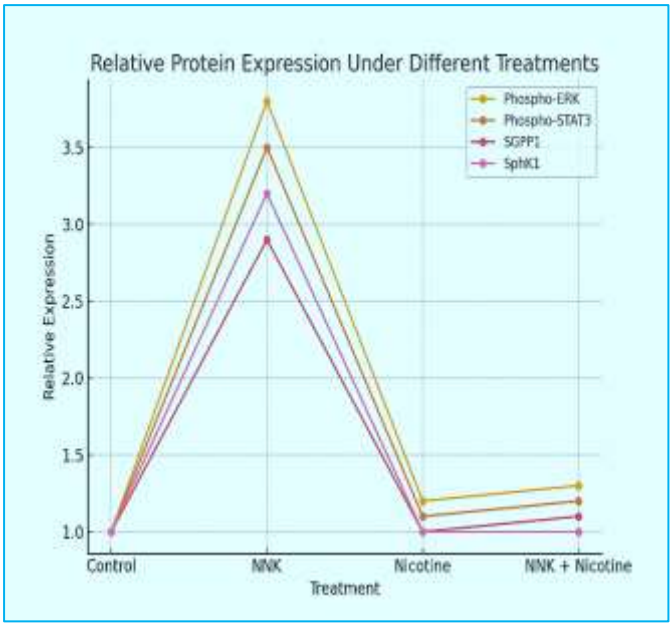

Figure 1. Relative expression levels of key signaling proteins in L-132 cells under various treatments.

DISCUSSION

The results confirm that NNK is a potent activator of the ERK and STAT3 pathways in L-132 lung cancer cells, aligning with previous reports on its carcinogenic properties. The downstream activation of SphK1 and SGPP1 highlights the involvement of the sphingosine-1-phosphate pathway in mediating oncogenic signals.

Nicotine, despite its prevalence in tobacco, demonstrated minimal impact on these pathways alone, possibly due to its non-carcinogenic but growth-promoting properties. The lack of signaling activation in the combination treatment could suggest interference by nicotine with NNK's signaling mechanisms, warranting further investigation.

CONCLUSION

This study underscores the differential impact of tobacco-derived compounds on lung cancer cell signaling. NNK acts as a potent inducer of oncogenic signaling pathways in L-132 cells, whereas nicotine shows limited activation. The surprising inhibitory effect observed in the combined treatment opens avenues for more nuanced studies into compound interactions within tobacco smoke.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest

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