FEATURED PUBLICATION

# The tobacco-specific carcinogen-operated calcium channel promotes lung tumorigenesis via IGF2 exocytosis in lung epithelial cells

This study presents novel mechanistic insight into tobacco carcinogen (NNK)-induced lung tumor formation through its effect on $Ca^{2+}$ signaling. It is hypothesized that NNK-mediated $Ca^{2+}$ influx will be suppressed by treatment with the pharmacological and genomic antagonists. Measurement of intracellular calcium levels was assessed using live cell time-lapse imaging and analysis with an Operetta® High Content Screening system; and *in vivo* tumor formation in mice treated with NNK and amlodipine or nifedipine was monitored using the IVISense™ MMP 680 fluorescent probe and the IVIS® SpectrumCT *in vivo* imaging system. This preclinical data suggest the use of calcium signaling blocking drugs as chemopreventive agents in smokers. This is in concurrence with a retrospective study of a public health database in which an inverse correlation between use of calcium channel blockers and lung cancer diagnosis was observed.

The phenotypic signatures and dynamics of cell signaling processing at the single cell level can be rapidly identified by imaging and measured using time lapse kinetics with the Operetta HCS imager. In this study, a consistent inhibition of NNK-induced intracellular calcium levels using a fluorescent reporter in HBEL/p53i primary cells and secretion of GFP-IGF2 in BEAS-2B immortalized cells seeded in 96-well Cell Carrier microplates were rapidly identified and quantitated.

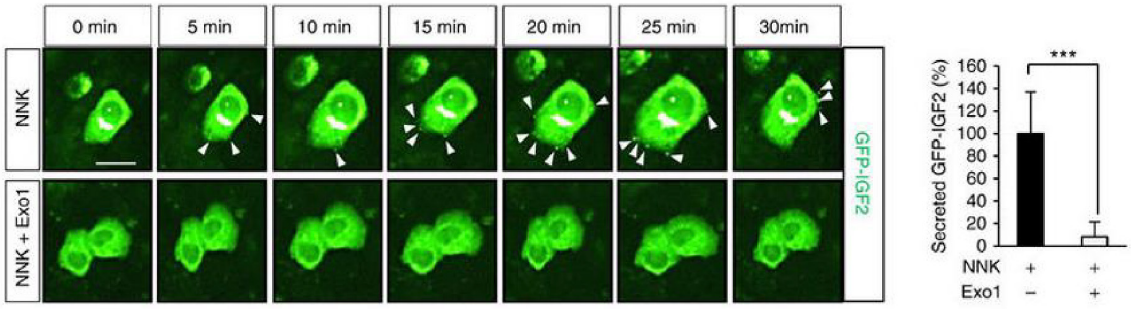
## Publication authors

Drs. Hye-Jin Boo, Hye-Young Min, Hyun-Ji Jang, Ho-Young Lee and colleagues

The Seoul National University, Seoul, Republic of Korea and The University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA

The figure on the top of page 2 represents the time-lapse images captured every 5 minutes over 30 minutes to show secretion of GFP-IGF2 (white arrows) in BEAS-2B cells following pre-incubation with Exo1 and NNK stimulus treatment. The findings are summarized in the bar graph showing a statistical significant difference between the Exo1 and NNK treatment groups.

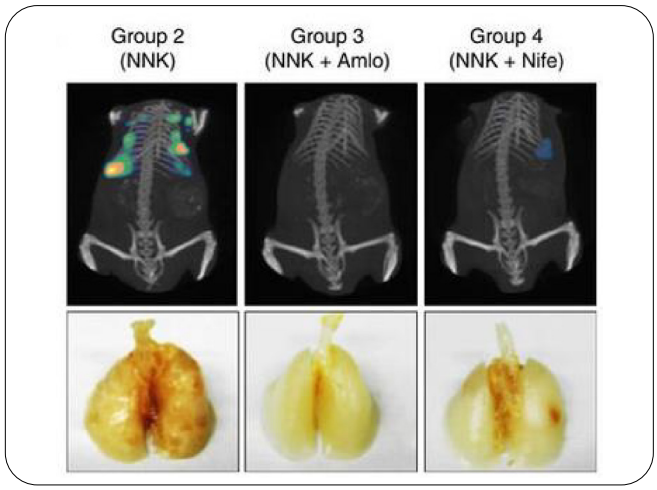
revvity




Matrix metalloprotease activatable fluorescent probe imaging may be used as a non-invasive lung tumor development assessment tool to validate the effectiveness of chemoprevention. IVISense MMP 680 is a protease activatable fluorescent *in vivo* imaging agent that is activated by key matrix metalloproteinases including MMP-2, -3, -9 and -13 released by cancer cells. IVISense MMP 680 is optically silent in its unactivated state and becomes highly fluorescent following protease-mediated activation. Three-dimensional (3D) fluorescence molecular tomography and fluorescent imaging probes are effectively used for *in vivo* detection of lung tumors and response to therapy.

FVB mice were untreated (group 1) or treated with NNK. The NNK-treated mice were subdivided into three groups: vehicle (group 2), amlodipine (group 3) or nifedipine (group 4) were administered 1 week after the first dose of NNK. Twelve weeks after the first dose of NNK, representative mice from each group were injected with the fluorescently activatable IVISense MMP 680 probe and imaged with the IVIS SpectrumCT to monitor lung tumor formation.

Lung tumors were easily detected in mice treated with NNK, but not in mice from the other groups. Gross evaluation of the lungs revealed no tumors in the lungs of control mice and 100% lung tumor formation in NNK-treated mice. In contrast, amlodipine- and nifedipine treated mice had obviously decreased lung tumor nodules.




In conclusion, Revvity translational drug discovery technologies helped reveal the mechanism of action and efficacy of calcium signaling blockers for chemoprevention initially in live lung cells, and subsequently in a mouse model of lung cancer. These observations were further found to correlate with a retrospective clinical study. This work elegantly showcases the power of streamlined translational drug discovery and development.

Boo et al. (2016 ). The tobacco-specific carcinogen-operated calcium channel promotes lung tumorigenesis via IGF2 exocytosis in lung epithelial cells. *Nature Communications 7:12961 | DOI: 10.1038/ncomms12961* © 2016 The Authors

**Revvity, Inc.**
940 Winter Street
Waltham, MA 02451 USA
(800) 762-4000
www.revvity.com

**For a complete listing of our global offices, visit www.revvity.com**