# Caffeic Acid Phenethyl Ester (CAPE), a natural radiosensitizer for lung adenocarcinomas

E. Prades Sagarra[1], R. Biemans[1], N.G. Lieuwes[1], P. Lambin[1], A. Yaromina[1], L.J. Dubois[1]

[1] The M-Lab, Department of Precision Medicine, GROW – School for Oncology and Reproduction, Maastricht University, Maastricht, the Netherlands.

## Abstract

Radiotherapy-induced adverse effects are dose limiting factors and narrow the therapeutic window. CAPE, an active component of propolis, has been suggested to widen the therapeutic window by having cytotoxic effects in tumor cells while protecting the normal tissue against radiation damage. CAPE has been shown to have anti-inflammatory and anti-oxidant effects in normal tissue, while anti-proliferative and pro-apoptotic effects in tumor cells. The aim of this study is to investigate the radiosensitizing effect of CAPE in a panel of lung cancer cell lines.

Viability of human adenocarcinoma (H1299, H1975, H522, HCC827) and non-adenocarcinoma (H520, H292) NSCLC lines was assessed after 24-hour incubation with increasing doses of CAPE using alamarBlue-based viability assays. Cell cycle analysis was performed after CAPE treatment by BrdU/PI staining. The effect of CAPE on metabolic profiles was determined using the Seahorse XF96 extracellular Flux analyzer. JC-10 assay was performed after CAPE treatment to assess mitochondrial damage. Clonogenic survival assays were performed after incubation with CAPE ($IC_{25}$ or $IC_{50}$ dose based on viability assays) followed by irradiation to assess its radiosensitizing properties.

Treatment with CAPE decreased cell viability in all lung cancer cells in a dose-dependent manner. $IC_{50}$ values varied between 32 and 88 μM. Cell cycle analysis showed an S-phase arrest after incubation with CAPE in majority of cell lines. Metabolic profiling showed a reduction in OCR while an increase in ECAR in all cell lines after CAPE treatment, suggesting that CAPE shifts cellular respiration towards glycolysis. CAPE also caused mitochondrial membrane depolarization, suggesting cell death due to an altered mitochondrial function. Clonogenic survival assays showed significant ($p<0.01$ or less) radiosensitization by CAPE in lung adenocarcinoma cell lines. Conversely, no significant differences were found in non-adenocarcinoma lines.

In conclusion, CAPE has cytotoxic effects in human lung cancer lines by altering cell cycle as well as cell metabolism and mitochondrial function. CAPE sensitizes lung adenocarcinoma lines to radiation, but not non-adenocarcinoma lines. To explain these differences, the study of the molecular mechanisms of CAPE-mediated radiosensitization in tumor cells, such as NF-κB pathway blockage and changes in oxidative stress, is ongoing. Furthermore, the radioprotective effects of CAPE in normal tissue are being investigated.