EVALUATION OF Δ9-TETRAHYDROCANNABINOL (THC) AND CANNABIDIOL (CBD) ON AN *IN VITRO* TRIDIMENSIONAL MODEL OF HUMAN LUNG CANCER

Irene Monleón-Guinot (1,2), Víctor Pérez (1), Morgane Millot (1), María Sancho-Tello (1,2), Jose Javier Martín de Llano (1,2), Carmen Carda (1,2,3), Lucía Bravo-Baranda (1,2), Irene Navarro-Esclapés (1), Mauro Llop-Miguel (1), Lara Milián (1,2) and Manuel Mata (1,2,3)

1. Departamento de Patología, Facultad de Medicina y Odontología, Universitat de València, Valencia, Spain; 2. Instituto de Investigación Biomédica INCLIVA, Valencia, Spain; 3. CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Spain.

Introduction

Previous research has highlighted the inhibitory effects of cannabinoid agonists on key factors of lung cancer, such as cell proliferation and epithelial-mesenchymal transition (EMT) [1,2]. However, the majority of these studies rely on two-dimensional *in vitro* models that do not consider the tumor microenvironment (TME), which is critical in lung cancer's metastasis and resistance to treatment. Cancer-associated fibroblasts (CAFs) play an important role in tumor progression via EMT, as they have the capability to secrete diverse factors, including transforming growth factor beta (TGF-β).

The aim of this study is to evaluate the effect of two cannabinoid agonists, Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), on a three-dimensional *in vitro* model. This model consists of spheroids formed by A549 cells and organoids of A549 combined with either CAFs or normal fibroblasts (NFs), supplemented with TGF-β.

Methods

Spheroids and organoids were formed using the hanging drop method and subsequently embedded in a rat type I collagen hydrogel. After an incubation period of four days, they were either supplemented with 5 ng/ml TGF-β or left untreated, and then treated with the cannabinoid mixture (THC + CBD, 10 µM each) or left untreated, followed by an additional three days of incubation. The spheroids/organoids were subsequently fixed in 4% paraformaldehyde. Immunofluorescence staining was performed to analyze cytokeratin filaments, F-actin was evaluated using rhodamine-conjugated phalloidin, and cell nuclei were stained with DAPI. Finally, the spheroids/organoids were examined using a confocal microscope.

Results

Immunofluorescence analysis showed a consistent morphology in the A549 spheroids under normal conditions, except for those treated with TGF-β, which displayed some small protrusions. Organoids of A549 co-cultured with fibroblasts did not exhibit significant differences in morphology (Figure 1).

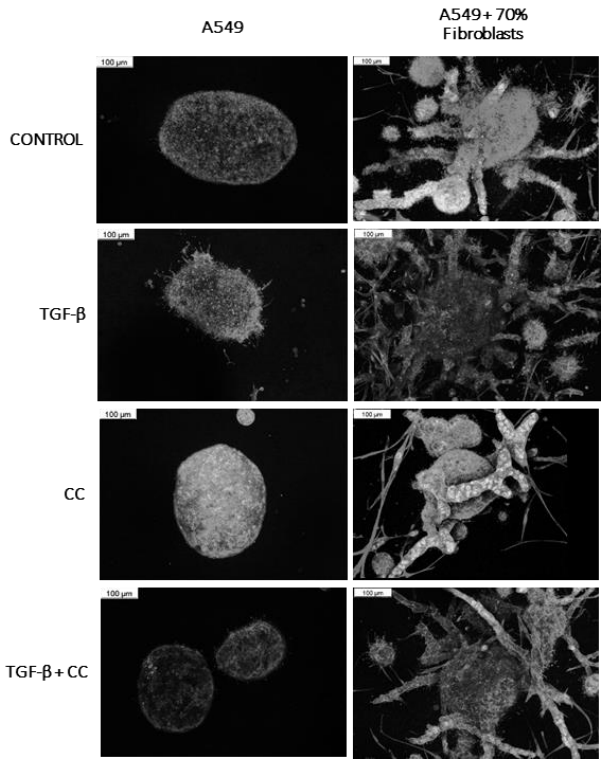

Figure 1: Morphology of human lung cancer organoids from each experimental condition.

Discussion

Our results suggest that while A549 spheroids were affected by TGF-β, organoids were not. This difference could be attributed to the endogenous expression of TGF-β in organoids, which may explain why their morphology remained unchanged. When cannabinoids were combined with TGF-β treatment, the phenotype induced by TGF-β in A549 spheroids was reversed. Furthermore, organoids did not show any morphological alterations. Overall, our results offer preliminary insights into the role of cannabinoid agonists in a 3D *in vitro* model of lung cancer.

References

1. Zang Y. et al, Oncol. Lett., 15:8527-8535, 2018.
2. Pacher P. et al, Annu. Rev. Pharmacol. Toxicol., 60:637-659, 2020.