L-Università ta' Malta

Department of Clinical Pharmacology and Therapeutics

Faculty of Medicine and Surgery

Targeting the PI3K/Akt/mTOR Pathway in Non-small Cell Lung Cancer Spheroids

Lung cancer is a major cause of death, with the non-small cell (NSCLC) subtype claiming a 15% 5-year survival rate. Deregulation of the PI3K/Akt/mTOR pathway and overexpression of a tumour-related protein, protein X, are classically occurring NSCLC events which both lead to eIF4E hyperactivity, therefore promoting tumour progression. We aimed to analyse the efficacy of AZD2014, a small molecule which inhibits the key pathway members, mTORC1 and mTORC2, in three NSCLC cell line models which are representative of the three main NSCLC subtypes. Additionally, effects of gene *X* knockdown was studied. Cells seeded in 2D cultures, were treated with various concentrations of AZD2014, and transfected with different amounts of a gene *X*-targeting antisense oligonucleotide (ASO), independently and in combination. Cell viability was analysed at 24, 48, 72 and 96h post-treatment. The results obtained were subsequently translated to spheroid 3D cultures, since these are more biomimetic models. NSCLC spheroid models were developed and characterised by studying their morphological characteristics, cell viability and formation of necrotic and proliferative areas over eight days in culture. Spheroids of each cell line were studied in terms of their size, cell viability and ATP production for up to six days post-treatment. AZD2014 alone did not induce a significant decrease in cell viability. However, a significant inhibition of spheroid growth and a decrease in cellular ATP levels was observed. These results point towards a cytostatic role of AZD2014 in NSCLC and highlights the significance of exploiting this novel combinatory therapy, which is currently being further studied.