DOTTORATO DI RICERCA IN ONCOLOGIA E CHIRURGIA SPERIMENTALI- INTERNAZIONALI- XXIX CICLO

Targeting PAM signaling pathway in the treatment of pancreatic ductal adenocarcinoma

INTRODUCTION:

Pancreatic ductal adenocarcinoma (PDAC) is among the most lethal solid tumors. Despite extensive preclinical and clinical research, the prognosis of this disease has not significantly improved, with a 5-year survival rate around 7%. This dismal outcome can partially be explained by the lack of biomarkers for screening and diagnosis at earlier stages, and by the resistance to most currently available chemotherapy regimens. This resistance has been attributed to both the desmoplastic tumor microenvironment and to the strong inter- and intra-tumor heterogeneity in terms of complexity of genetic aberrations and the resulting signaling pathway activities, as well as to resistance mechanisms that quickly adapt the tumor to drugs. As a consequence, there is an urgent need to better understand the molecular pathology of PC in order to improve patient selection for current treatment options, and to develop novel therapeutic strategies.

PI3K/Akt/mTOR (PAM) pathway is often altered in patients with cancer. This pathway controls several biological activities within the cell, and its activation is one of the fundamental downstream molecular events following tyrosine kinase growth factor receptor activation.

In pancreatic ductal adenocarcinoma (PDAC) PAM pathway plays a crucial role because deregulation of components involved in this pathway could confer resistance to chemotherapy, while blockage of Akt signaling results in programmed cell death and inhibition of tumor growth. Activation of Akt is a frequent event in PDAC and has been correlated to its poor prognosis.

Several inhibitors of Akt are under investigation, but three are the farthest along and showed the most promise in early clinical research: the pan-Akt and PI3K inhibitor perifosine (KRX-0401,

Aeterna Zentaris/Keryx), the allosteric pan-Akt inhibitor MK-2206 (Merck), and the dual PI3K/mTOR inhibitor dactolisib (NVP-BEZ235, Novartis).

In particular, the synthetic oral alkylphospholipid perifosine has been evaluated in clinical trials for several tumors, including colon, breast, head and neck and prostate cancer. Unfortunately, it failed the phase III clinical trials for treatment of colon cancer and relapsed refractory multiple myeloma (www.clinicaltrials.gov). These failures together with the disappointing response rates to perifosine as a single agent in most solid tumors, including PDAC, prompt further studies into its mechanism of action as well as on synergistic combinations.

AIM:

The aim of my project is to investigate the expression of phospho-Akt in PDAC tissues and cells, and to evaluate the effects of growth inhibition by Akt inhibitors, using PDAC cell lines and primary cultures growing as monolayer or as spheroids. Moreover, characterize several key factors, affecting cell cycle perturbation, apoptosis induction, as well as inhibition of cell migration and invasion and modulation of key factors in glucose metabolism in PDAC cells exposed to perifosine and perifosine/gemcitabine combination.

MATERIALS AND METHODS:

Drugs and chemicals, cell culture, growth inhibition studies, tissue microarrays, immunohistochemistry (IHC) and immunocytochemistry (ICC), quantitative reverse-transcriptase polymerase-chain-reaction (QRT-PCR), western blotting (WB), evaluation of synergistic/antagonistic interaction with gemcitabine, effects on multicellular spheroids, akt and phospho-akt analysis by enzyme linked immunosorbent (ELISA) assay, in vitro migration and invasion assays, analysis of cell-cycle and cell death, caspasi activity assay, analysis of modulation of glut1 by flow cytometry, evaluation of the cytotoxic and pro-apoptotic effects inhibition of glut1 inhibition combined with akt inhibitors, statistical analysis.