ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancers and accounts for 7 % of cancer-related mortalities worldwide. The disease has an overall 5year survival rate of less than 5 % and a median survival of 6 months, with mortalities caused by the disease being almost equal to the incidence each year. The poor prognosis associated with PDAC is attributed to its mostly asymptomatic nature and lack of specific biomarkers in the early stages of the disease, thus most cases are diagnosed at an advanced stage where metastasis has already occurred. Despite perceived improvements in chemotherapeutic and biological agent strategies, the prognosis of advanced PDAC remains dismal. This study investigated the role of the insulin-like growth factor 1 receptor (IGF-1R) pathway in PDAC *in vitro.* The baseline expression the IGF-1R and downstream signalling was determined in control and PDAC cell lines, which represented normal, early-, intermediate and late-stages of the disease. Further assessment of the role of IGF-1R signalling was assessed through modulation of the pathway with IGF-1, a stimulatory compound; and NVP-AEW541 and Picropodophyllotoxin (PPP) IGF-1R inhibitors. Diluent controls of DMSO and BSA were also used.

The HPDE, PANC-1. Mia PaCa-2 and CF PAC-1 cell lines were used. These cell lines are representative of the tumours from which they were derived, i.e., PANC-1, Mia PaCa-2 and CF PAC-1 cells represent the early, intermediate, and late stages of PDAC, and HPDE cells represent a normal, healthy pancreatic ductal cells. The cytotoxic potential of the NVPAEW541 and Picropodophyllotoxin inhibitors were determined using the Trypan blue exclusion method. The WST-1 assay was used to compare proliferation between the cell lines. Clonogenic and migration phenotypic assays were used to evaluate the survival and migratory capacity of the PDAC cells. Alterations in gene expression was assessed using quantitative real-time polymerase chain reaction (qRT-PCR). In Mia PaCa-2 cells, protein expression of downstream members of the IGF-1R pathway was assessed using a western blot analysis technique.

Across all cell lines the IC_{50} of NVP-AEW541 was found to be between 2.5 µM and 2.7 µM, and between 0.65 µM and 4 µM for the Picropodophyllotoxin inhibitor. NVP-AEW541 and Picropodophyllotoxin was found to significantly decrease cell proliferation in the HPDE, PANC-1 and CF PAC-1 cell lines. In addition, HPDE cells appeared to be the most sensitive cell line to the different treatment groups when compared to the other cell lines. Colony counts were significantly decreased in all cell lines when treated with the DMSO diluent, with Picropodophyllotoxin being more effective than NVP-AEW541 in preventing large colony formations. Migration was significantly decreased in all cell lines when treated with DMSO, NVP-AEW541 and Picropodophyllotoxin. Analysis of gene expression data of genes involved in the IGF-1R pathway showed that NVP-AEW541 increased the expression of these genes in HPDE and CF PAC-1 cells. In PANC-1 and Mia PaCa-2 cells the expression of these genes was downregulated in cultures treated with NVP-AEW541.

Picropodophyllotoxin either decreased or had a minimal effect on gene expression in all cell lines. Protein expression of IGF-1R was found to be constant across all treatment groups in the Mia PaCa-2 cell line.

This study found that even though IGF-1R was not a predominant signalling cascade in the Mia PaCa-2 cell line which represents the intermediate stage of PDAC, and it is hypothesized that the cytotoxic effects of the compounds used may have been metabolised by these cells at a later point. It was further shown that PDAC cells responds differently at different stages of its progression, showing that a single treatment for all stages of the disease is not sufficient in improving overall patient survival. This demonstrates an urgent need for molecular subtyping of tumours which may lead to more tailored therapies for PDAC.