ABSTRACT

Gemcitabine, an effective agent in the treatment of cancer of pancreas, has failed many times after multiple cycles of therapy due to drug resistance. An effective therapeutic approach to treat pancreatic tumors, refractory to gemcitabine therapy, has emerged in the form of combination of dietary compounds with clinically validated drugs. In the present study to optimize a possible synergistic combination of Gemcitabine (GCB) with dietary molecules, Betulnic acid (BA) and Thymoquinone (TQ), stand-alone IC$_{50}$ dose of GCB, BA and TQ was calculated for pancreatic cancer cell lines. Fixed IC$_{50}$ dose ratio of the dietary molecules in combination with reduced IC$_{50}$ dose of GCB was tested on GCB resistant PANC-1 and sensitive MIA PaCa-2 cells for synergism, additive response and antagonism, using calcsyn. Combination index (CI) revealed that pre-treatment of BA and TQ along with GCB synergistically inhibited the cancer cell proliferation in in-vitro experiments. Pyruvate kinase (PK) M2 isoform, a promising target involved in cancer cell metabolism, depicted down-regulation in presence of TQ or BA in combination with GCB. GCB with BA triggered mitochondrial permeability transition by acting preferentially on tumor mitochondria. Pre-exposure of MIA PaCa-2 and PANC-1 cell lines to TQ in combination with GCB induced apoptosis. The effectiveness, therefore, of BA or TQ in combination with GCB to inhibit cell proliferation, induce apoptosis and down-regulate the expression of PKM2, provides promise in treating pancreatic cancer. To assess the therapeutic potential in two pancreatic cancer cell lines we investigated the combination effect of microRNA, nutraceuticals and drug (MND). MIA PaCa-2 and PANC-1 cells transfected with miR-101 or miR-24-2 were treated with Betulinic acid or Thymoquinone and gemcitabine independently and in combination and assessed for the extent of synergism in both experimental and control conditions,
considering significance at the p value of <0.05. miR-101 or miR-24-2 over-expressing cells when treated with lower than IC_{50} doses of the dietary compounds and drug showed a reduced (37–50%) viability in two cell lines with differential synergistic effect and the outcome for Pro-caspase3, Poly (ADP-ribose) polymerase (PARP) cleavage and PKM2 expression. Hence, the two independent microRNA backgrounds showed promise in therapeutic intervention of gemcitabine sensitive, MIA PaCa-2 and resistant, PANC-1 pancreatic cancer cells, in combination with dietary agents and drug.