ANTITUMOUR EFFICACY OF CHYMOTRYPSINOGEN AND TRYPSINOGEN

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INTRODUCTION

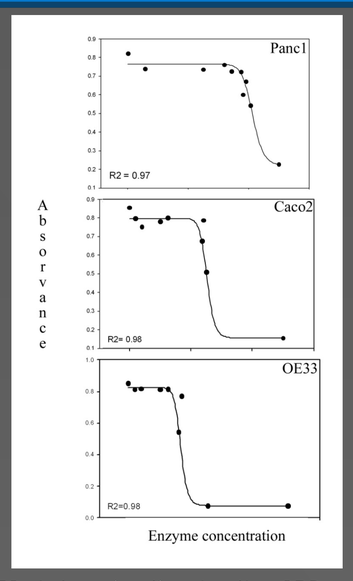
Tumour metastasis represents a major cause of cancer related deaths. Cell detachment, migration and invasion of the surrounding tissues are key events in this process. Consequently, there is an urgent need to develop new therapeutic approaches to treat metastatic cancer. **Previous research has suggested a utility of pancreatic (pro)enzymes in cancer treatment. These observations started on 1960s when John Beard observed that fresh pancreatic juices inhibited cancer growth in vitro**

AIMS

The aim of the present study is to investigate *in vitro* the effects of a mixture of two pancreatic pro-enzymes, i.e., Chymotrypsinogen and Trypsinogen (PRP), on the growth, migration, angiogenic potential differentiation and epithelial-mesenchymal transition (EMT) characteristics of ovarian, colon and pancreatic human cancer cell lines.

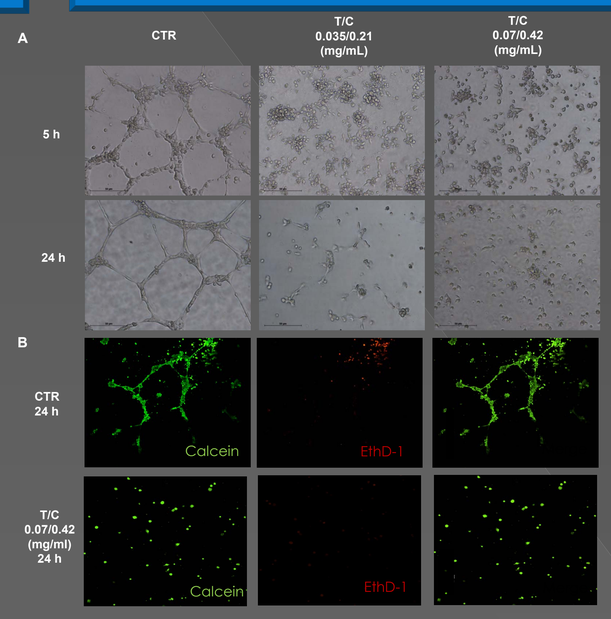
RESULTS

Growth Inhibition

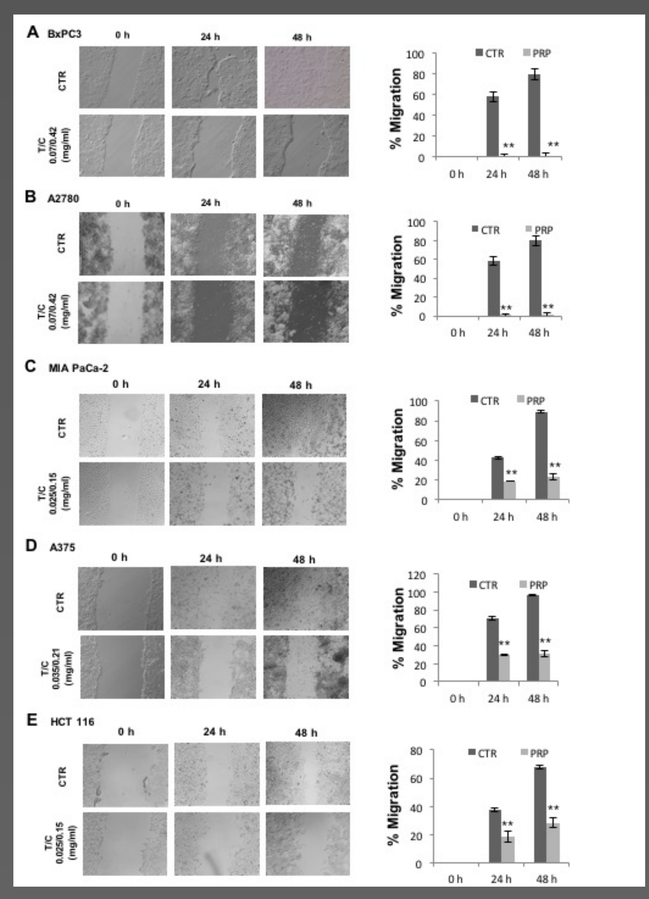


IC50 determination by the SRB assay in Panc1, Caco-2 and OE33 cells

Angiogenesis and Migration Inhibition



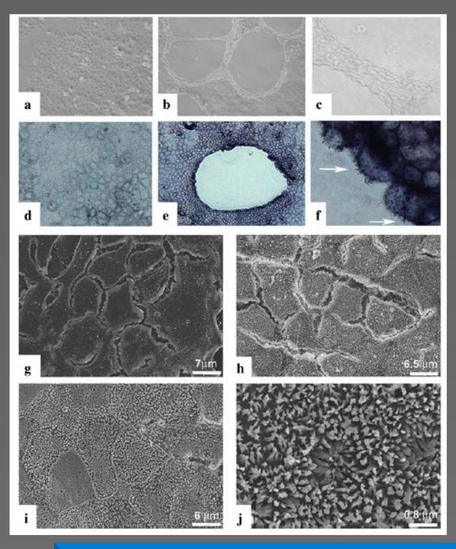
Angiogenesis tube-formation assay.
HUVEC cells were grown on Matrigel and the formation of capillary-like structures was assessed
Cell viability assay (B) by Calcein AM.
Original magnification 10x for all panels.



Wound healing assay to determine cell migration (A) performed at 0, 24 and 48 h in BxPC3, A2780, MIA PaCa-2, A375 and HCT 116 cells in IC 50 treated cells and untreated cells used as controls.

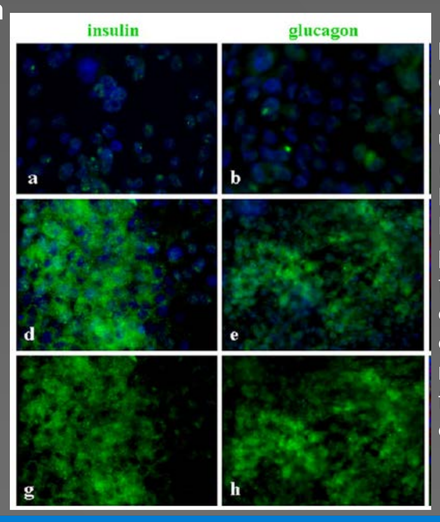
% Cell migration (B) was determined by the rate of cells moving towards the scratched area upon time using ImageJ™ (∗P < 0.05 and ∗∗P < 0.01).

Cell Differentiation



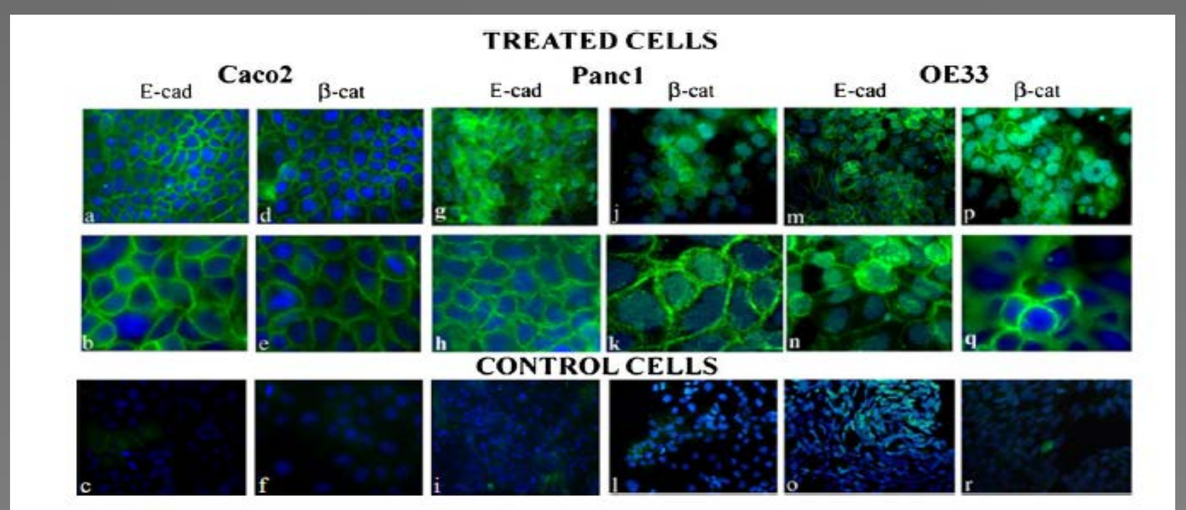
Caco-2 and Panc1 cells treated with (pro)enzymes exhibit lineage-specific differentiation

Phase-contrast microscopy showing highly confluent monolayers of untreated (a and d) and treated (b, c, e and f) Caco-2 cells. Scanning electron micrographs of untreated (g) and treated (h–j) cells show erected microvilli. In (j) an enlargement of (i) is shown. Representative pictures from two independent experiments are shown. Original magnifications: 20 x (a, b and e), 40 x (d), 63 x (c) and 100 x (f)

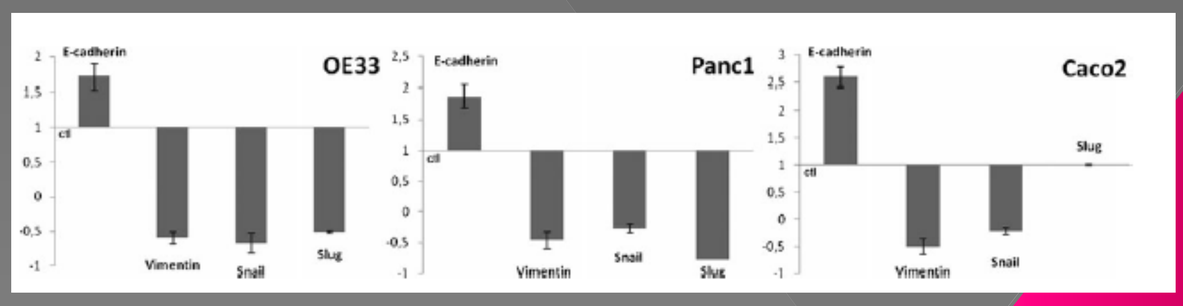


Immunofluorescent detection of insulin and glucagon in untreated (a,b) and (pro)enzyme treated Panc1 cells (d-h). Representative pictures from three independent experiments are shown. Original magnification 40 x for all the panels

EMT Related Markers



Epithelial marker expression in Caco-1, Panc1 and OE33 cells treated with (pro)enzymes. Cell surface E-cadherin and β-catenin expression was detected (in green) in fixed and permeabilized cells, respectively. The images show the membrane distribution of these markers. The original magnification was 40 x for all the panels, except b, e, h, k, n and q (63 x)



Real-time PCR analyses of EMT gene expression in OE33, Panc1 and Caco-2 cells. Gene expression is shown as fold change relative to control cells cultured in medium without (pro)enzymes (ctl) normalized to 1.0. Data are shown as average ± SD (n=3) from at least 2 independent experiments

CONCLUSION

Our results show that the treatment of human cancer cell lines with pancreatic (pro)enzymes results in an enhancement of cell adhesion, an attenuation of several EMT-associated markers, and an increase in the expression of several differentiation-associated markers, suggesting the acquisition of a less malignant phenotype and a decrease in proliferative capacity due to lineage specific cellular differentiation. Furthermore, the treatments showed inhibition of angiogenesis when proven on HUVEC cells by matrigel-based tube formation.