The role of diabetes and microRNA in pancreatic cancer

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MicroRNAs, (miRNAs, miRs) represent class of small non-coding RNAs closely associated with the post-transcriptional control of gene expression at mRNA level. miRNAs are single-stranded molecules containing approximately 18-24 nucleotides, and are responsible for regulating the expression of nearly 60% of all protein-coding genes. Pancreatic cancer is an aggressive tumor, diagnosed at a late stage. It is characterized by a high mortality rate and limited treatment strategy. Diabetes is characterized by chronic high levels of glucose in the blood.

Research plan

1. Impact of hyperglycemia on the viability of cells: PANC-1, 1.2B4, HPNE (COMPLETED FOR PANC-1 AND HPNE, IN PROGRES FOR 1.2B4 cells).

2. Screnning of 753 microRNA expression relative to the U6 reference gene with significantly changed expression in cell lines:PANC-1 and HPNE cultured in normoglycemia and hyperglycemia. (COMPLETED FOR PANC-1 AND HPNE).

3. Identification of the mRNA molecules and signaling pathways involved in the microRNA molecules selected in point 2 of the research plan by conducting bioinformatics analysis. COMPLETED FOR PANC-1, IN PROGRES FOR HPNE).

4. Study on the expression of microRNAs selected in point 2 of the study plan and targeted mRNAs and associated proteins in:

a) Cell lines: PANC-1, 1.2B4, HPNE cultured under normoglycemic and hyperglycemic conditions

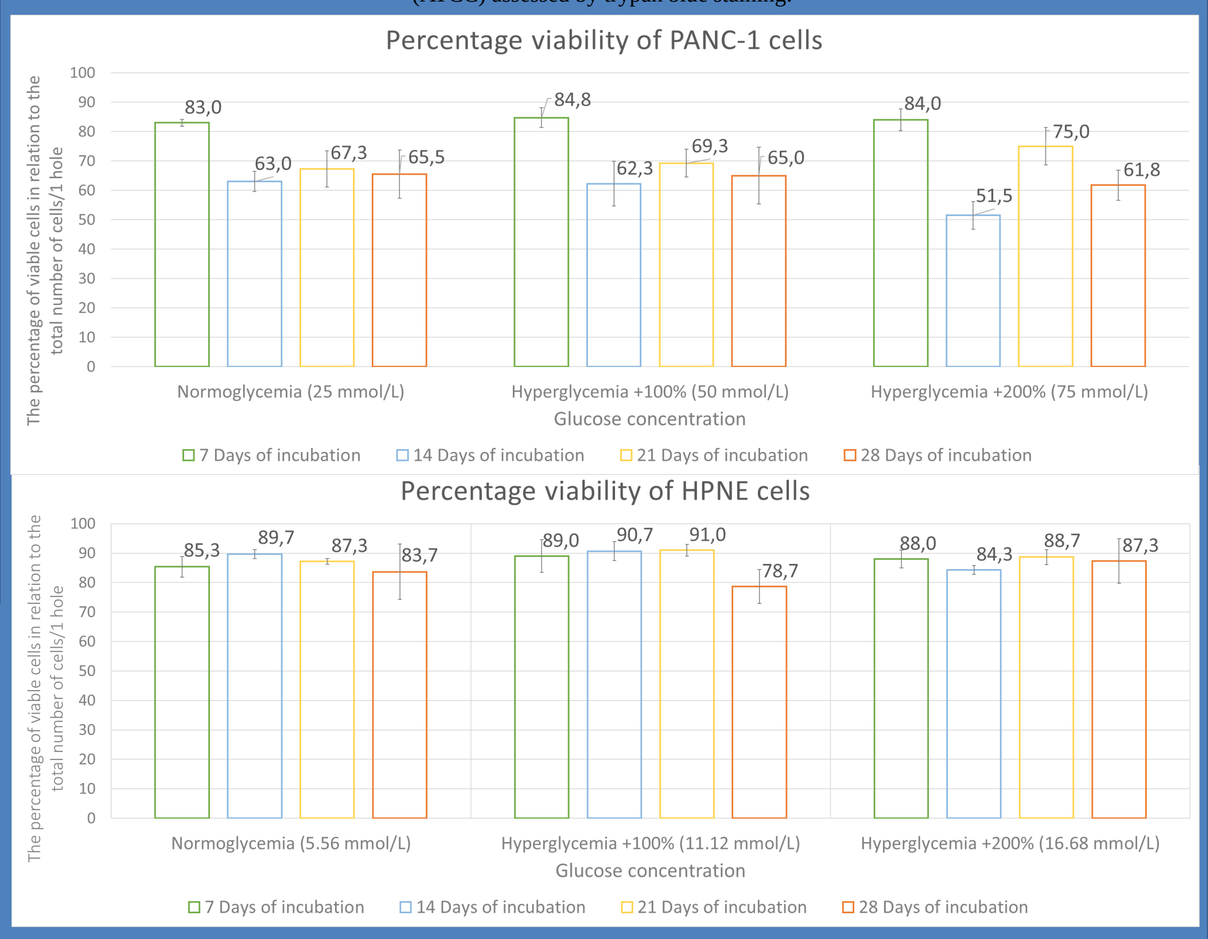
b) Tumor tissue collected from patients with pancreatic cancer, with and without co-existing diabetes.

c) Plasma of pancreatic cancer patients with and without co-existing diabetes.

Obtained results

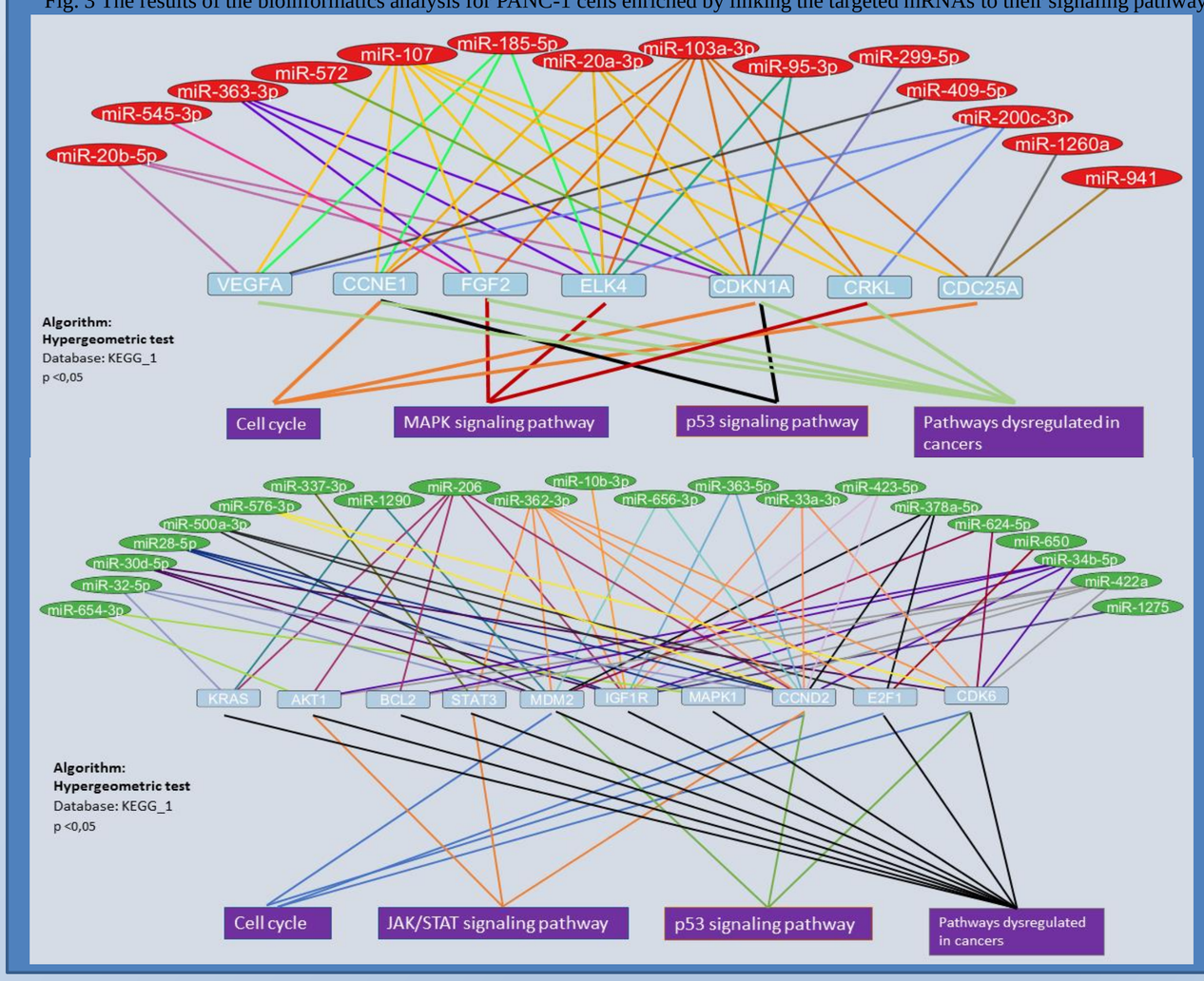
The effect of hyperglycemia on viability PANC-1 and HPNE cells

Fig. 1 Effect of hyperglycemia on HPNE and PANC-1 cell viability relative to the manufacturer's recommended glycaemia (ATCC) assessed by trypan blue staining:



In silico analysis of dysregulated microRNA by hyperglycemia in PANC-1 cells

Fig. 3 The results of the bioinformatics analysis for PANC-1 cells enriched by linking the targeted mRNAs to their signaling pathways:



The impact of hyperglycemia on micoRNA profile in PANC-1 cells

Fig. 2 Effect of hyperglycemia on microRNA expression profile - down-regulated microRNA molecules in the PANC-1 cell line (miRNA screening):

	microRNAs
Up-regulated	miR-20a-3p, miR-20b-5p, miR-95-3p, miR-103a-3p, miR-107, miR-185-5p, miR-193a-3p, miR-200c-3p, miR-299-5p , miR-671-3p , miR-720 , miR-941 , miR-1260a , miR-1227-3p, miR-1300, miR-363-3p, miR-369-5p, miR-375-3p, miR-409-5p, miR-545-3p, miR-572, miR-616-3p
Down-regulated	let-7a-5p, miR-7-1-3p, miR-10b-3p, miR-15a-3p, miR-28-5p, miR-30d-5p, miR-32-5p, miR-154-3p, miR-362-3p, miR-422a, miR-500a-3p, miR-555, miR-656-3p, miR-1275, miR-1290, miR-33a-3p, miR-34b-5p, miR-206, miR-337-3p, miR-363-5p, miR-378a-5p, miR-423-5p, miR-483-5p, miR-517c-3p, miR-576-3p, miR-581, miR-624-5p, miR-650, miR-654-3p, miR-1204

Conclusions

1.The results indicate that there are no significant changes in cell viability under hyperglycemic conditions. A small decrease of viability in the PANC-1 cell line was observed for glycaemia 200% 14th day in relation to normoglycemia exposure.

2. In the PANC-1 cell line, 22 miRNAs were up-regulated (RQ >2) , and 30 miRNAs were down-regulated (RQ <0.5) under hyperglycemic conditions.

3. Bioinformatics analysis showed that the identified microRNAs target KRAS, AKT1, BCL2, STAT3, MDM2, IGF1R, MAPK1, CCND2, E2F1, CDK6, VEGFA, CCNE1, FGF2, ELK4, CDKN1A, CRKL, CDC25A in PANC-1 cell line, these genes are involve in the following signaling pathways: MAPK, JAK/STAT, p53, pathways related to cell cycle and apoptosis.

4. I have currently collected 2 tumor tissue samples and 2 plasma samples from pancreatic cancer patients.

Aim of the research

As microRNA expression can be tested in blood, cells, tissues and extracellular fluid, it could to be a good candidate for non-invasive diagnostic biomarker. My study focus on epigenetic control of gene expression associated with exposure to chronic hyperglycemia, a symptom that accompanies diabetes and which is a factor in the development of pancreatic cancer. Therefore, the aim of this study is the role of microRNAs and diabetes in pancreatic cancer. My study plan will allow for identification of key signaling pathways and the molecules involved in these pathways, in vitro. Furthermore, analyses performed in the plasma of pancreatic cancer patients with and without co-morbid diabetes, diabetes-only patients and patients with normal glycaemia will allow an assessment of the importance of diabetes as a risk factor for pancreatic cancer. The expression of miRNAs will also be tested in the tumor tissue of patients divided into 2 groups: without and with co-morbid diabetes, to compare the in vitro and in vivo model.