The study of the anti-cancer, epigenetic mechanisms of action of lunasin in human leukemia cells

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Introduction

The aim of the planned doctoral dissertation is to investigate the anti-cancer properties of lunasin - a protein obtained from soybeans. It is planned to perform cultures of human leukemic cells and normal human peripheral blood cells with lunasin in various concentrations, as well as molecular research aimed at assessing methylation and expression of selected genes encoding epigenetic enzymes that control cellular functions, including those involved in neoplastic transformation.

Cancer is one of the main causes of death in the world, so it is necessary to look for new ways to prevent it. Epigenetic modifications play an important role in the process of carcinogenesis, including leukemia. Plant-derived natural compounds, which include lunasin, may modulate the occurrence of epigenetic processes in cells. Therefore, it is necessary to conduct studies to assess the effect of lunasin on the expression of genes encoding epigenetic enzymes and DNA methylation to determine lunasin's potential in the chemoprevention of leukemia.

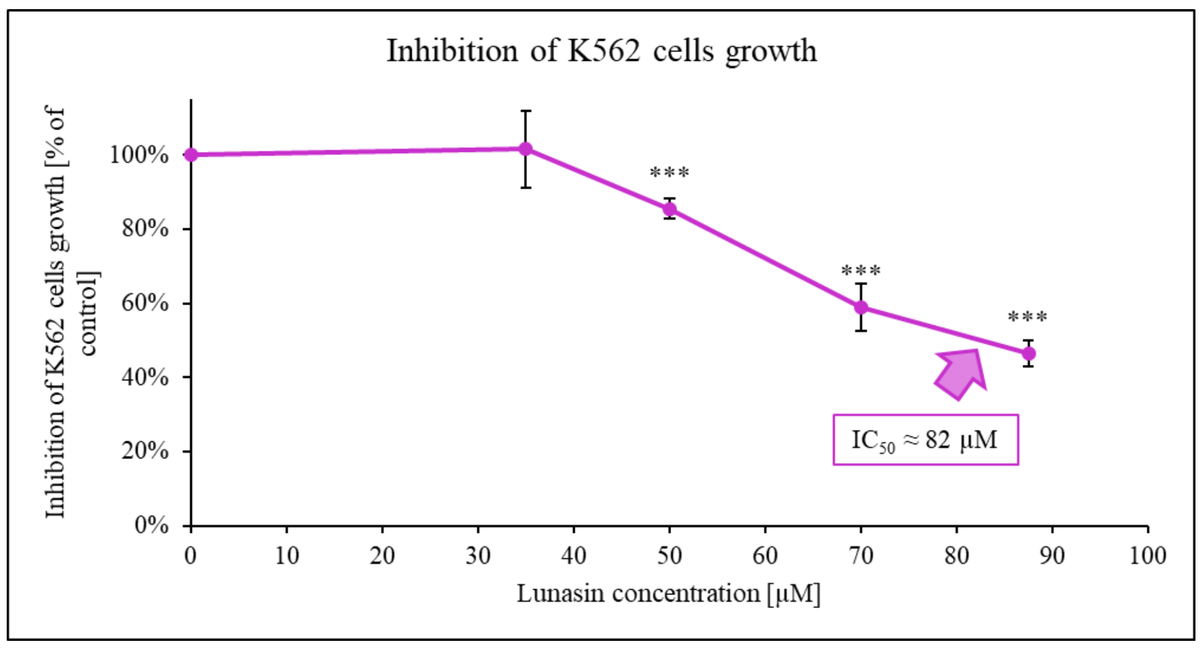
Research hypotheses

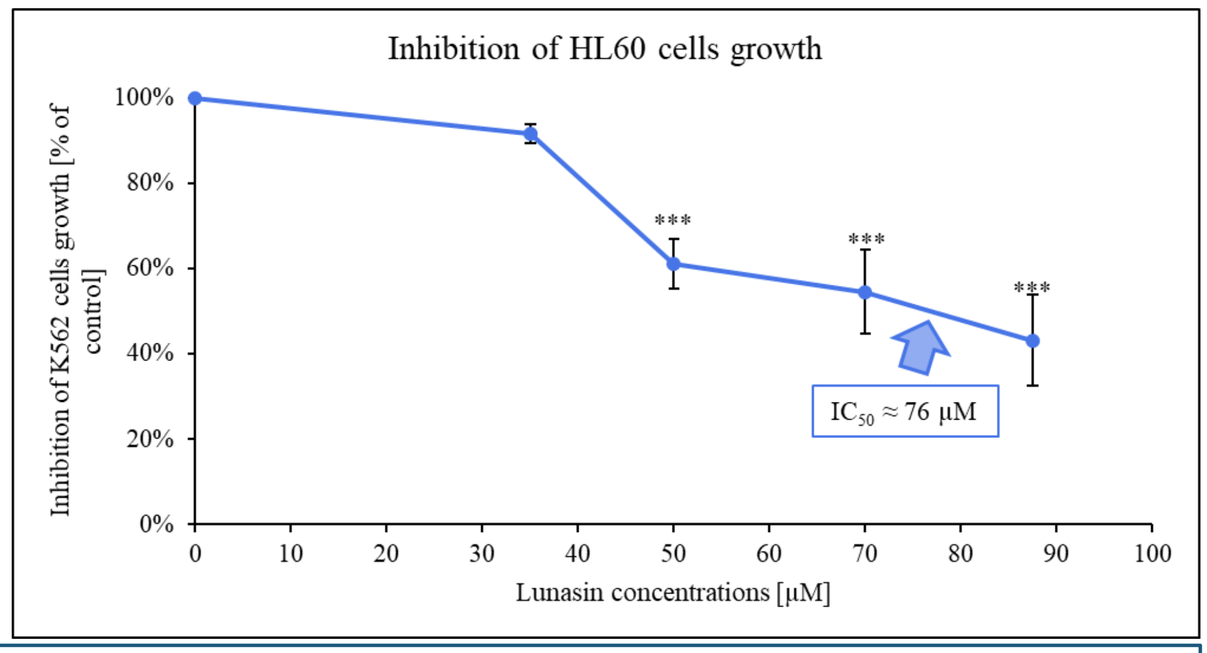
1. Lunasin has an anticancer effect - it inhibits the growth and proliferation of human leukemia cells
2. Lunasin is selective for cancer cells and has no negative effect on normal human cells
3. Lunasin causes a change in the expression of genes encoding epigenetic enzymes in leukemic cells
4. Lunasin changes the DNA methylation profile in leukemic cells

Matherials and methods

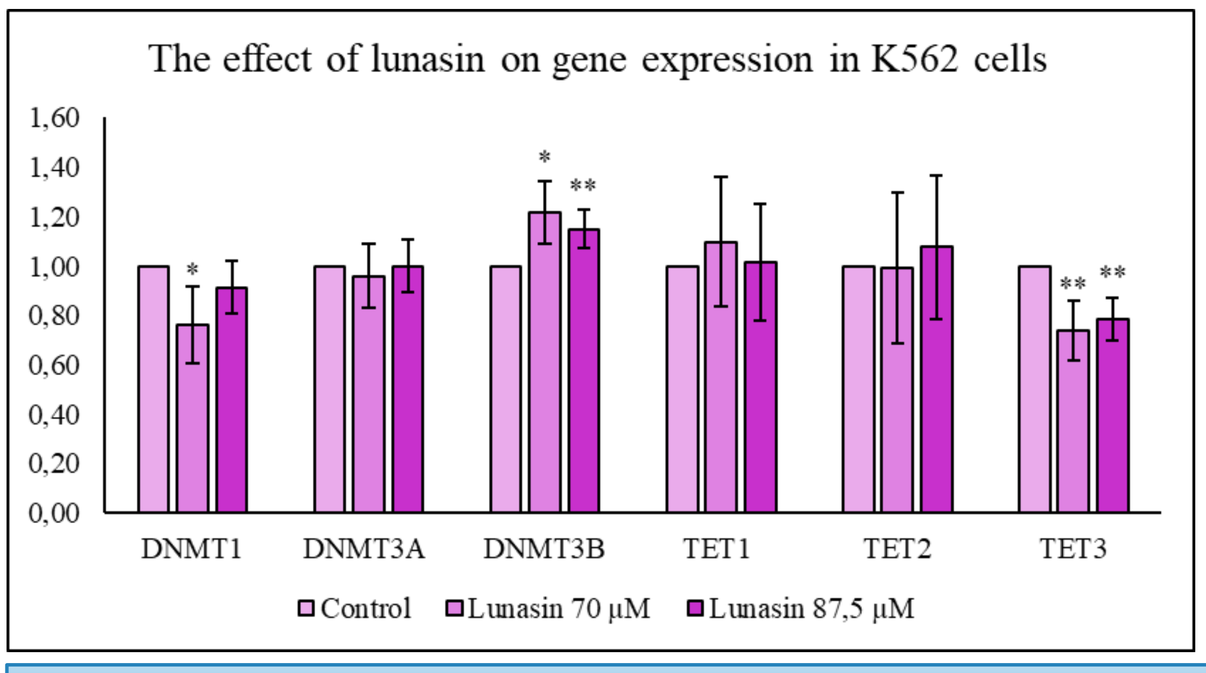
- Cell culture of K562 and HL60 cell lines in RPMI 1640 culture medium with lunasin at concentrations of 70 and 87.5 µM i four separate experiments.
- Evaluation of cell growth inhibition by lunasin by microscopic counting of cells stained with trypan blue using a Fuchs-Rosenthal cell.
- Isolation of RNA from control and tested cells using the Trizol reagent. Spectrophotometric evaluation of RNA purity.
- Synthesis of complementary DNA (cDNA) by reverse transcription - GoScript™ Reverse Transcription System (Promega).
- Evaluation of the expression of the tested genes by real-time polymerase chain reaction (real-time PCR) - GoTaq® qPCR Master Mix (Promega).
- Statistical analysis of data obtained in the real-time PCR reaction using the Pfaffl method.

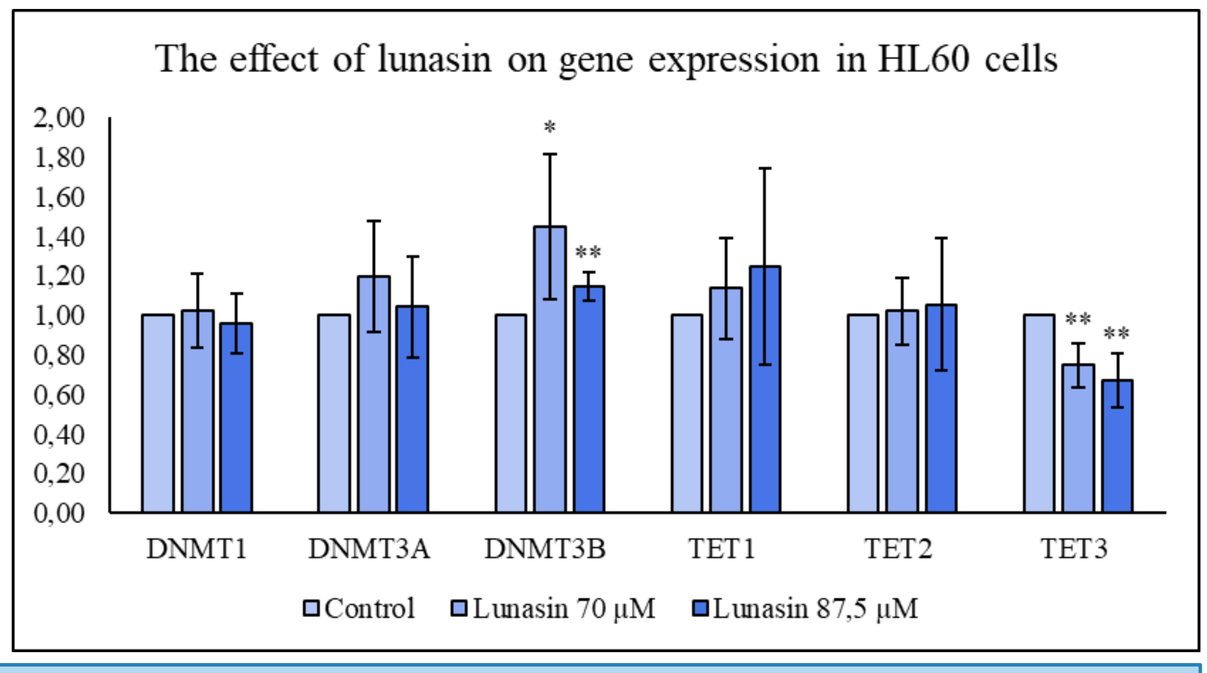
Results





- Lunasin at concentrations of 70 and 87.5 µM inhibited cell growth by 41 and 54% in K562 cells and by 45 and 57% in HL60 cells, respectively. Statistical analysis of obtained results indicated their statistical significance ($p<0.001$). Necrotic cells constitute < 10% of cells.
- Gene expression was affected by lunasin. This year's research was primarily focused on genes involved in DNA methylation (*DNMT1*, *DNMT3A*, *DNMT3B*) and demethylation (*TET1*, *TET2*, *TET3*).
- There was a statistically significant increase in expression of *DNMT3B* and decrease in *TET3* expression in both cell lines.





Conclusions and future plans

Based on the obtained results, it can be concluded that lunasin has anticancer properties. It inhibits the growth of K562 and HL60 leukemia cells, and also affects changes in the expression of genes involved in epigenetic modifications and tumor suppressor genes. However, further research is needed. Planned research at further stages of the preparation of the doctoral dissertation includes, among others, assessment of cell proliferation after culture with lunasin using the MTT method, assessment of the effect of lunasin on the growth of normal human cells, assessment of DNA methylation in leukemic cells after culture with lunasin and extension of the profile of the tested genes to include genes related to the apoptosis process (*CASP3, BAX, BCL2*).