EXAMINATION OF GENE EXPRESSION OF MOLECULAR MECHANISMS OF MIGRATION, APOPTOSIS AND REDOX STATUS OF COLORECTAL CARCINOMA CELLS

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Abstract:

Colorectal cancer (CRC) is one of the leading causes of mortality worldwide, the fourth most common cause of cancer-related death. Understanding the mechanisms of migration, invasion, metastasis, and testing for new bioactive substances is one of the strategies in treating cancer. This study aims to investigate the relative gene expression profile in colorectal carcinoma cells in treatment with chemotherapeutics. The results of our research are based on three groups of genes for the cytoskeleton: *E-cadherin, β-catenin* and *N-cadherin*; for redox status: *HIF1A, Nrf2, NFkB, TXNRD, GADPH, GST, GPX1, GPX2, GPX3* and *GPX4;* and for apoptosis: *Caspase 3, Caspase 9, Caspase 8, BAX, Fas,* and *BCL-2*. All three groups of genes showed different gene expression after treatment with different concentrations of chemotherapeutics compared to untreated tumor cells, which served as controls. The use of chemotherapeutics in the treatment of cancer is reflected in the consideration of gene expression in order to find the most precise concentrations and doses that will affect the apoptosis of tumor cells.

Keywords: migration, redox status, chemotherapeutic, apoptosis, ANOVA

1. Introduction

Colorectal cancer is one of the major malignancies and the second most common cancer type [1]. This type of cancer is one of the main diseases in developed countries and it mostly develops spontaneously because there is no known hereditary predisposition [2]. The colorectal malignancies mainly spread from adenomatous polyps [3]. Hence, understanding the mechanisms of molecular features such as migration, invasion and apoptosis is crucial [4]. Cancer cells spread from the primary tumor to a distant organ and, to manage that, they need to undergo migration, invasion and intravasation into the blood/lymphatic vessels. Molecules involved in that pathway could be potential targets for anti-cancer therapy [5].

2. Instructions

2.1. Cells treatment

The chemotherapeutics (5-fluorouracil 50 mg / mL, oxaliplatin 5 mg / mL, irinotecan 40 mg / mL, leucovorin 50 mg / mL) were diluted to operating concentrations (10, 50, 100 μM). After confluence dosing, the medium was aspirated from the bottle and the cells were treated with different concentrations of cytostatics.

2.2. Sample preparation and total RNA isolation

Total RNA was isolated using the manufacturer's instructions. After 24 h, RNA was isolated from the cell using a spin colony kit for RNA purification. The kit contains all the necessary reagents to isolate and protect RNA from RNase. Samples with RNA were eluted (impurities were removed by washing) and kept in RNase-free water at -20 °C.

2.3. Reverse transcription and cDNA synthesis

Master Mix was prepared for reverse transcription, containing Reaction Buffer, MgCl2, Deoxynucleotide Mix, Oligo-dT Primer, RNase Inhibitor, and Reverse Transcriptase. Master Mix, PCR grade water and RNA samples were added in a total volume of 20 μL. The total RNA was subsequently reverse transcribed, resulting in cDNA synthesis during the second incubation and reaction was performed using MIC qPCR Cycler (Biomolecular Systems, Yatala, Australia). cDNA was diluted with RNase-free water for qRT-PCR reaction.

2.4. Relative expression of miRNA using qRT-PCR method

Obtained cDNA was mixed with PCR grade water, forward and reverse primers previously designed, control RNA, template RNA and Master Mix 2 (containing PCR grade water, RT-PCR buffer, Enzyme mix). MIC qPCR Cycler was used for cycling reaction with additional high-resolution melting analyses. Melting analyses were performed at the end of 40 cycles as the proof of reaction product procedure. Every qPCR run was also analyzed with an NTC control reaction.

To calculate the relationship between cytostatic treatments and the expression of these genes in control or untreated cells, the following formula is used:

$$\Delta\Delta\boldsymbol{CC}_{\mathrm{T}} = \Delta\boldsymbol{CC}_{\mathrm{T}}\mathbf{11} - \Delta\mathbf{CC}_{\mathrm{T}}\mathbf{22}$$
$$\mathbf{22}^{-\Delta\Delta\boldsymbol{CC}_{\mathrm{T}}} = \boldsymbol{RR}$$

2.5. Statistics

The data were expressed as mean ± standard error (SE). All qPCR reactions were performed in triplicates. Statistical significance was determined using the Student's t-test or the oneway ANOVA test for multiple comparisons. The magnitude of correlation between variables was done using SPSS (Chicago, IL) statistical software package (SPSS for Windows, version17, 2008).

3. Results

Analysis of gene expression of enzymes important for the cytoskeleton (Figure 1) changes significantly in the treatment with 5-fluorouracil (10 and 100 μM) and leucovorin (50 μM). For genes notable in redox control, the expression changed after treatment, especially with 5-fluorouracil (10 and 100 μM), which induces increased expression of most redox equilibrium genes. Also, expression was the most increased after treatment with leucovorin (10 and 50 μM), and in a few genes with oxaliplatin. Gene expression of enzymes significant in apoptosis (Figure 2) was also altered after treatment with 5-fluorouracil (10 and 100 μM) for all genes except for Bax and BCL2.

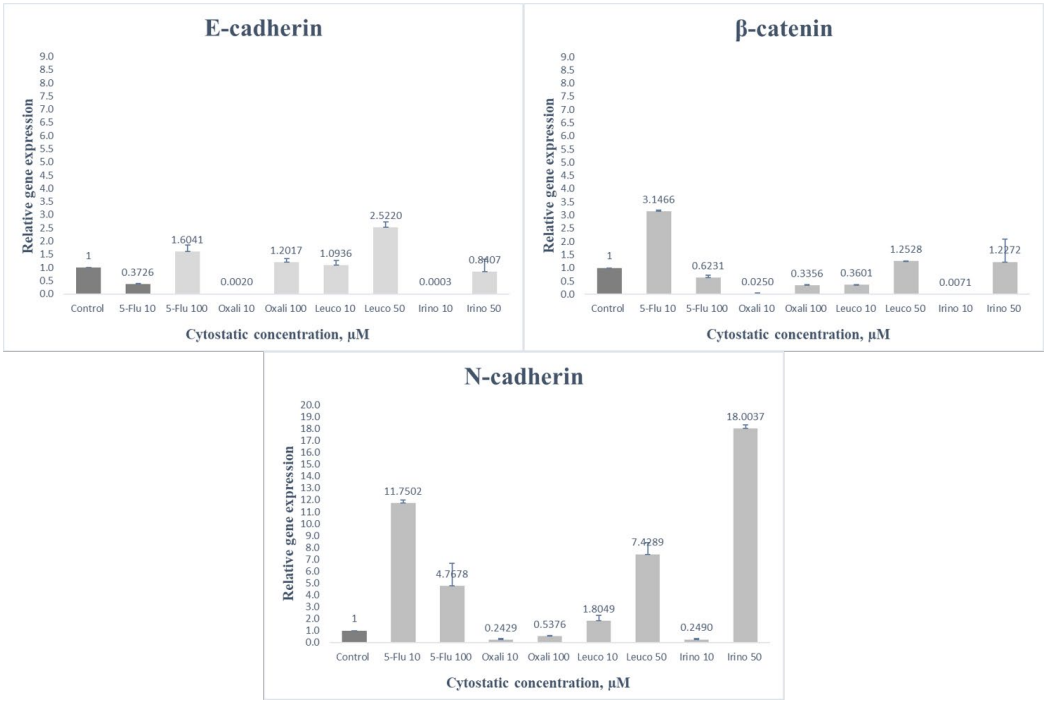

Fig. 1. Relative expression of E-cadherin, N-cadherin and β-catenin genes in the treatment of cytostatics on HCT-116 cell line

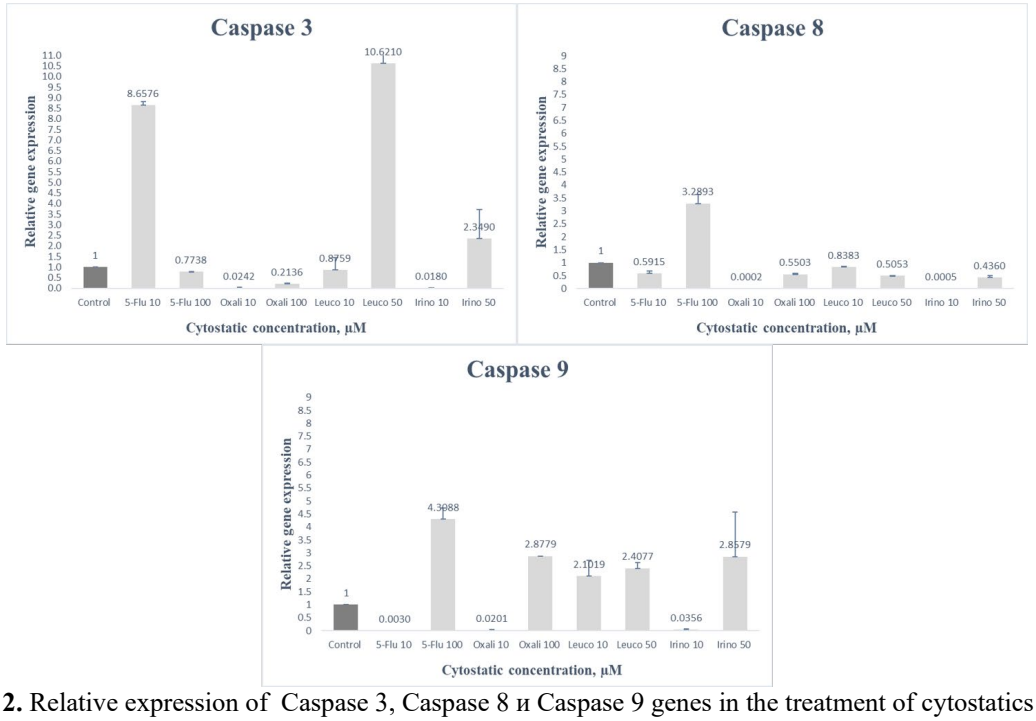

Fig. 2. Relative expression of Caspase 3, Caspase 8 и Caspase 9 genes in the treatment of cytostatics on HCT-116 cell line

4. Conclusion

The limitation of this study is the small number of experiments. Given a larger number of experiments, future research will focus on building a classification model that tries to draw conclusions from the input values given for training. It will predict whether therapy with chemotherapeutics will be successful using molecular parameters of tumor cells as input

parameters (class labels (<1, >1)/categories for the new data). For that purpose, algorithms, logistic regression, K-Nearest Neighbours, Decision Tree, Support Vector Machine etc. will be used.

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