Studying the impact of 5-FU in colon cancer cells in comparison with colon cancer stem cells

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Background: 5- fluorouracil (5-FU), an analog of uracil known as anti-metabolite is a widely used anticancer prodrug that, after administration, is intracellulary converted into its main active metabolites (FdUMP, FdUTP and FUTP). Is thought to be cytotoxic to tumor cells by three potential mechanisms: inhibition of thymidylate synthase (TS) by FdUMP ; incorporation of FdUTP into DNA ; and incorporation of FUTP into RNA. Colorectal cancer (CRC) is one of the most common human cancers, for which 5-FU is usually part of the treatment. Growing evidences indicate that tumors such as CRC initiate from a small part of cancer stem cells (CSCs). This scientific approach attempts to prove if there is a difference between colon cancer cell lines and colon cancer stem cell lines after their treatment with 5-FU.

Materials & Methods: In order to prove the above hypothesis commercial colon cancer cell lines and colon cancer stem cell lines were used. After treatment with 100 μM 5-FU for 24h, total RNA was extracted and Reverse Transcription (RT) and Real – Time PCR assays, with specific primers for each marker were conducted.

Gene	Primer sequence (5'->3')
TYMS	Forward:TCTGCTGACAACCAAACGTGTGTTC
	Reverse: CCATTGGCATCCCAGATTTTCAC
DHFR	Forward: AGTCAGCGAGCAGGTTCTCATTGA
	Reverse: TGGACTATGTTCCGCCCACACA
SHMT1	Forward:CCAGAGATACTATGGCGGGACTGAG
	Reverse:CCAGCACTGTGGGTCCAGCTTATAG
DPYD	Forward: AGGAGGGTTTGTCACTGGCAGACT
	Reverse: TTCTTGGCCGAAGTGGAACACAG

Table 1: Primer sequence for genes studied

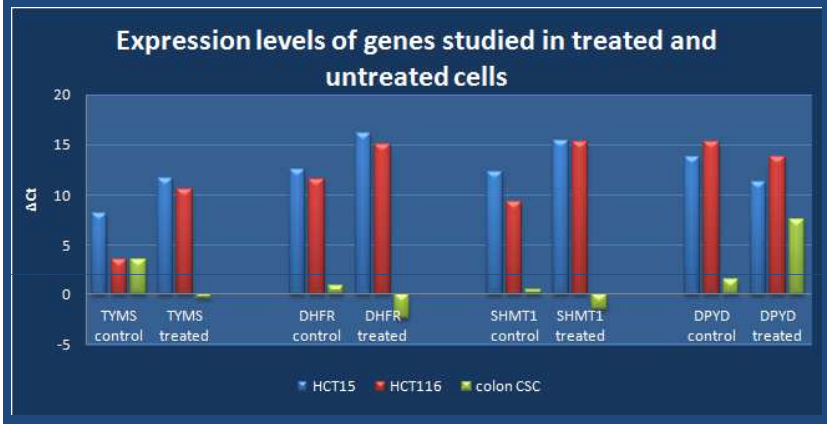

Figure 1: Real- Time PCR results by using specific markers

Results: The 18S rRNA was used as an internal control for quantitative analysis. After treatment, TYMS, DHFR and SHMT1 expression seems to be reduced while DPYD seems to be increased in both colon cancer cell lines (HCT- 15 and HCT- 116). In the colon CSC line the opposite expression pattern is observed as TYMS, DHFR and SHMT1 expression is increased and DPYD is reduced.

Conclusion: The CSC hypothesis suggests that rare populations of tumor-initiating cells may lead, among others, to resistance to therapy. This hypothesis is supported by the previous data, since it is clearly demonstrated that colon CSCs respond differently to 5- FU treatment compared to colon cancer cells.

Selected References:

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