Correlation between FOLFOX regimen and stemness in colon CSCs

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Background: The most recommended line of therapy in advanced colon and colorectal cancer is FOLFOX (5-FU in combination with oxaliplatin). However, it is said that this chemotherapeutic model is insufficient and is associated with stemness. Because colon CSCs (cancer stem cell – like cells) have been proven to be resistant to chemotherapy, increased proliferation and expansion of them may be correlated with incomplete response and cancer recurrence by following the above mentioned therapeutic strategy. The present assay was performed in order to examine whether a correlation between FOLFOX influence and colon CSCs exists.

Method: In order to prove the above hypothesis, two different methods have been chosen. As the nanog gene is the basic stemness marker, a siRNA - based method was used for repressing it in FOLFOX – treated and FOLFOX – untreated cells. FOLFOX was at a concentration of 50 μM 5-FU + 1.25 μM oxaliplatin). The second panel of the test included a RT - qPCR – based protocol in order to test the expression of three transcription factors (nanog, oct3/4, and sox2) by using gene specific primers and 18S rRNA as endogenous gene. It has been indicated by previous studies that this triplet plays a crucial role in the cascades which promote stemness. For the above experiments, two different populations of colon CSCs were used and cultivated by using the appropriate growth media. The first population was isolated from patients who suffered from colon carcinoma and the second was a commercial colon cancer stem cell like – cell population. The analysis has been performed by using relative quantification normalized to the reference gene, according to Livak’s method.

Results: It has been shown that when nanog’s gene expression was reduced by 20% (repression of nanog gene by siRNA – based method) in both FOLFOX – treated and FOLFOX – untreated colon CSCs, the expression of the other two markers was varied. Specially, according to Livak’s method, the oct3/4 (transfected cells) / oct3/4 (non transfected cells) ratio was 2 in FOLFOX – treated cells and 2.68 in FOLFOX – untreated cells. Concerning the sox2 gene, the ratio was 2.80 and 30.38, respectively. Although the expression of both oct3/4 and sox2 was increased post nanog knock down, there was a greater increase the expression of sox2 gene in untreated cells. This means that these two markers may act synergistically, as a dimer, in stemness pathway. (fig. 1 & 2). The figure 3 represents the viability of cells after 48h of incubation post knock down which was almost the same in all populations (non – transfected cells, transfected cells with FOLFOX and transfected cells without FOLFOX) (fig. 3). Concerning the phenotype of cells, the greater difference was between transfected and non transfected cells than in treated in comparison with the untreated (fig 4a – 4e).

Conclusion

The present scientific attempt indicates that the FOLFOX, a well-established chemotherapeutic agent, may be correlated with stemness which, in this particular case, has been transiently influenced.