

## Effect of manuka honey on 5-fluorouracil chemosensitivity in colonspheres enriched with cancer stem (-like) cells

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### Abstract

Manuka honey (MH) has an *in vitro* chemosensitizing effect against 5-Fluorouracil (5-Fu) in colonspheres enriched with cancer stem (-like) cells (CSCs-like); these interesting findings, although preliminary, suggest a potential synergistic action of MH with 5-Fu, which could be useful in deepening the studies on natural compounds to be used in association with conventional medical therapies.

### Introduction

Colorectal Cancer (CRC) represents worldwide the 3<sup>rd</sup> cancer for incidence and the 2<sup>nd</sup> for mortality.<sup>1</sup> The low efficacy of the pharmacological treatments used, among which 5-Fu is the most common, is largely attributable to the phenomenon of chemoresistance typical of a rare tumor subpopulation: the CSCs.<sup>2</sup> In recent years, research has been focusing on the study of various compounds, including natural ones, that have a chemosensitizing effect on common chemotherapeutic drugs. In this context, honey represents a natural food product known to modulate various biological activities and prevent various diseases, including cancer.<sup>3</sup> The main objective of the present work was to evaluate the *in vitro* effect of MH and its combination with 5-Fu on CSCs-like deriving from colon adenocarcinoma cell line, enriched through the sphere formation assay, a 3D tumor model that better mimics what really happens *in vivo*.<sup>4</sup>

### Materials and Methods

Two different cell lines were used: human colon adenocarcinoma cell line and human colon fibroblast cell line.

The ability of MH alone and in combination with 5-Fu to decrease some morphological and physical parameters of colonspheres enriched with CSCs-like.

The effect of MH alone and in combination with 5-Fu on ROS intracellular accumulation and apoptotic rate in colonspheres enriched with CSCs-like was evaluated with cytometry.

Western Blot and Real-Time PCR analysis were used to evaluate the effect of MH alone and in combination with 5-Fu on colonspheres enriched with CSCs-like regarding different peculiar characteristics and processes of CSCs.

Self-renewal and migration assay were used for evaluating the effect of MH alone and in combination with 5-Fu on colonspheres enriched with CSCs-like on self-renewal ability and migration capacity.

Statistical analysis was performed using STATISTICA software (Statsoft Inc., Tulsa, OK, USA). For the significant differences are used letters that were acquired using one-way analysis of variance (ANOVA) followed by Tukey's honest significant difference post hoc test ( $p < 0.05$ ).

### Results

- MH alone and more in combination with 5-Fu was able to decrease some morphological and physical parameters of colonspheres without exerting any cytotoxic effect on healthy colon cells.

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- MH and its combination with 5-Fu induced intracellular accumulation of ROS associated with increased apoptosis rate.
- MH in combination with 5-Fu was able to downregulate some gene expression strictly associated with a higher chemoresistance.
- MH was able to enhance the effect of 5-Fu in the downregulation of one of the main targets of 5-Fu.

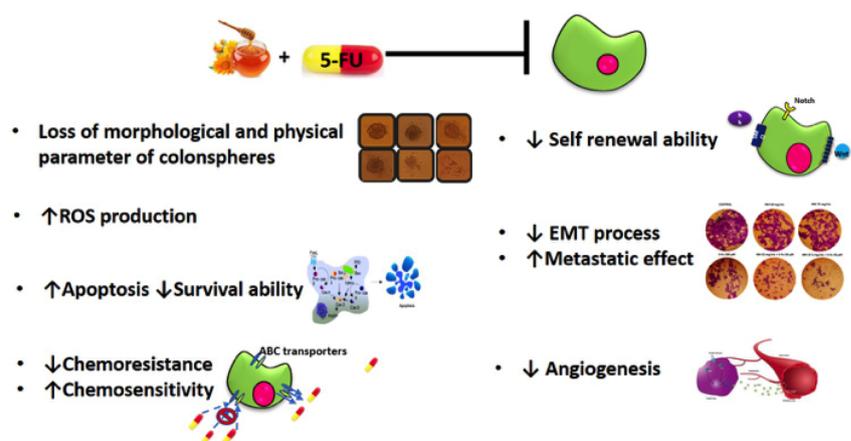


Figure 1. Summary of the effects showed by MH and 5-Fu on colon CSCs-like.

- MH was able to increase the effect of 5-Fu on the self-renewal ability.
- MH, especially in combination with 5-Fu, was able to reduce the migration capacity of CSCs-like and some pro-angiogenic factors.

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## Discussion and Conclusions

These results indicate that MH has an *in vitro* chemosensitizing effect against 5-Fu on colon CSCs-like (Figure 1); these interesting findings, although preliminary, suggest a potential synergistic action of MH with 5-Fu, which could be useful in deepening the studies on natural compounds to be

used in association with conventional medical therapies which, in addition to increase the effectiveness of 5-Fu, also may decrease its side effects. The main limitation of this study is related to the bioavailability and bioaccessibility of phenolic compounds. This limitation can be a starting point for subsequent studies involving *in vitro* simulated digestion processes. The treatment with the digested fraction could allow an approach to a physiological condition closer to the real one.

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