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Bee product royal jelly suppress EMT and invasiveness of HCT-116 cells †

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Abstract: The most frequent type of cancer, colorectal cancer (CRC), is widely recognized as the most common cause of death worldwide, due to the high invasive potential of cancer cells enabling metastasis. Cancer cells owe these properties to epithelial-mesenchymal transition (EMT), which requires overexpressed markers Snail and vimentin. Considering that natural products have been intensively investigated from anticancer point of view, we aimed to investigate effects of royal jelly, natural bee product, on invasiveness of colorectal cancer cell line HCT-116 and expression of these two proinvasive/EMT markers. Our study reports on inhibited expression of Snail and vimentin in tested cells, due to which suppressed aforementioned potential was detected.

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1. Introduction

As the highly frequent type of cancer diagnosed in both males and females, colorectal cancer (CRC), is also widely recognized as the most common cause of death worldwide. Globally it is one of the cancers whose incidence is increasing, and the prognosis of cancer patients' survival is often poor due to the acquisition of invasive and migratory potential of cancer cells which consequently leads to metastasis [1]. Firstly, cancer cells succumb to specific process - epithelial to mesenchymal transition (EMT), acquiring invasive potential. This allows them to detach from primary cancer site, therefore, cells are enabled to penetrate in the surrounding stiff extracellular matrix. For this purpose, expression of certain markers that enable this transition is necessary to assess, such as Snail, nuclear protein, or cytoskeletal protein Vimentin. It is known that Snail expression starts in the first stage of colorectal carcinogenesis, whereat this transcriptional factor acts as repressor of many epithelial hallmarks, as well as potentiator of mesenchymal markers [2]. Hence, therapy designed to battle cancer from this point of cancer progression involves targeting aforementioned markers.

We aimed to investigate effects of royal jelly, natural bee product, originated from Serbia, on invasive potential of colorectal cancer cell line HCT-116 and expression of these two invasive EMT proteins.

2. Materials and methods

HCT-116 cells, isolated from rectal region of human colon carcinoma, were purchased as immortalized cell line from American Type Culture Collection (ATCC, Manassas, USA). Cells were cultured in complete culturing medium (Dulbecco's Modified Eagle Medium – DMEM, supplemented with 10% of Fetal Bovine Serum – FBS, and penicillin/streptomycin), in humified atmosphere, at 37°C and 5% of CO_2, and seeded for assays

at 80-90% of confluency. Royal jelly sample (produced by *Apis mellifera* L. species) was collected in Central region of Serbia and diluted fresh in Phosphate Buffer Solution (PBS) and complete cell culturing medium. This way two working concentrations 10 and 100 μg/mL were obtained and applied for all the following assays, while the effects of RJ was analyzed after 24 h.

Invasive potential of control (untreated) and HCT-116 cells treated with RJ was investigated using assay with Transwell inserts coated with thin collagen layer, which was appied as an extracellular matrix. This assay was performed as described earlier [3]. Absorbances were read using Multiskan SkyHigh Microplate Spectrophotometer (Thermo Scientific, USA) (595 nm wavelength) and results from three independent experiments performed in triplicates are presented in form of invasive index.

In order to assess the protein expression of Snail and Vimentin, immunofluorescent assay was performed in three independent experiments and in triplicates, as described earlier (Jovanovic et al., 2022). Inverted fluorescent microscope Eclipse Ti (Nikon Instruments Inc., Tokyo, Japan) was used to obtain micrographs (at 600× magnification) which were furtherly analyzed to quantify relative fluorescence intensity of targeted proteins. For this purpose, ImageJ software package was applied following the procedure described earlier [4].

Statistical comparison of obtained results was done by IBM SPSS statistical software package (NY, USA) using One-way Anova test. Results from all performed assays are presented as mean ± standard error, where $*p < 0.05$ designate a statistically significant difference between treatments and control, and $\#p < 0.05$ designate a statistically significant difference between effects of treatment concentrations.

3. Results

According to our results showed in Figure 1, the suppression of invasiveness of tested colorectal cancer cell line, HCT-116, was observed 24 h after treatment with two applied RJ concentrations (10 and 100 μg/mL). This suppression is obvious and statistically significant when compared to control values.

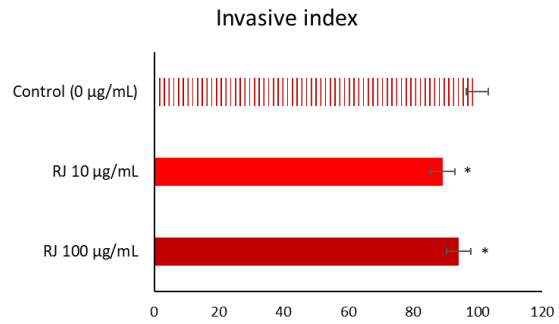

Figure 1. Invasive potential of control and HCT-116 cells treated with RJ in two selected concentrations (10 and 100 μg/mL) presented as mean values ± SE; $*p < 0.05$ is considered as statistically significant difference between treatments and control values.

In order to assess the possible mechanism of RJ's antiinvasive potential, the protein expression of regulatory factor Snail and effector marker vimentin was investigated using immunofluorescent method, and the results can be observed on representative micrographs, as well as in graphs (Figure 2). Firstly, the expression intensity of Snail in control (untreated) HCT-116 cells was at very high level, and the localization of this protein was in both cell nuclei and cytoplasm, indicating its abundance in cells (Figure 2 a). Meanwhile, the treatment was able to suppress it significantly and restrict its localization only to cell nuclei. In this suppression, the lower RJ concentration exerted more significant effect, than higher concentration applied, which correlates with its antiinvasive potential (Figure 1).

On the other hand, control (untreated) HCT-116 cells contained relatively high level of vimentin, found in both cell nuclei and cytoplasm. When treatment with RJ was applied, the significantly lower level of this protein expression was observed, which was mainly localized in cell nuclei (Figure 2 b). The lower RJ concentration had slightly stronger suppressive effect on this protein expression, when compared to higher RJ concentration. These results regarding vimentin protein expression also correlate with anti-invasive properties exerted by RJ, as showed earlier (Figure 1).

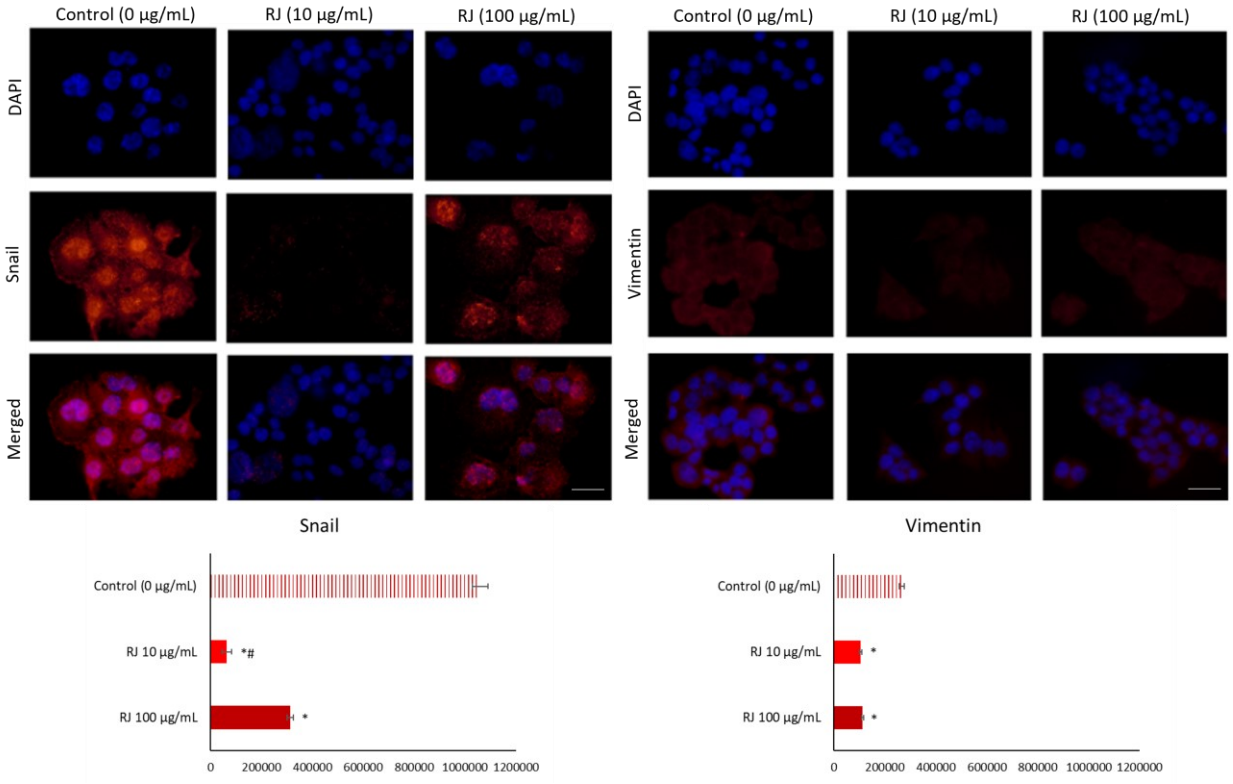

Figure 2. Representative micrographs showing Snail (a) and vimentin (b) fluorescence intensity in control and treated HCT-116 cells. Micrographs were obtained 24 after treatment with RJ in two selected concentrations (10 and 100 µg/mL). Results of relative protein fluorescence intensity are presented as changes compared to control values (mean ± SE), where $*p < 0.05$ is considered as statistically significant difference between treatments and control values, and $\#p < 0.05$ is considered as statistically significant difference between treatment concentrations.

4. Discussion

So far, the common treatments for colon cancer in clinics are chemoradiotherapy, surgery and immunotherapy. However, many disadvantages have been noticed, which is why scientists are turning to development of alternative types of therapeutic approaches to this disease. Natural products have been used in folk medicine for centuries, and many of them proved to be effective in treating cancer [5]. Royal jelly, as natural product, already showed to possess anticancer properties by suppressing cancer growth and aggressiveness [6]. Therefore, we aimed to assess antiinvasive potential of royal jelly sampled in Serbia, on colorectal cancer cell line HCT-116 which is already described as highly invasive and aggressive [7]. Therefore, the suppression of this cell behavior is highly desirable approach for designing anticancer therapeutics.

Present study report on suppression of invasiveness of tested colorectal cancer cells by treatment with RJ, obviously induced by lowered protein level of Snail and Vimentin. Previous investigations confirm that natural products possess the ability to repress levels of these two invasive markers *in vitro*, as well *in vivo* [8].

It is generally accepted that a fundamental characteristic responsible for formation of metastasis is acquisition of invasive potential and is associated with Snail and Vimentin expression [2]. Overexpression of Snail, as zing-fingered transcriptional factor, is responsible for resistance to chemotherapeutics, lymph node metastasis, activation of EMT program, stemness of cancer and poor prognosis of CRC patients [9,10]. Activation of Vimentin expression by Snail was already reported, resulting in increased invasive and migratory potential of cancer cells, enabling their dissemination (metastasis). Also, the occurrence of this nuclear factor has not been detected in normal (healthy) epithelial cells, however, it was found present in invasive front of cancer tissue [11]. The tight connection between two investigated EMT markers with significant role in acquisition of invasiveness in cancer cells resulted in suppression of this very undesirable trait of HCT-116 cell line.

5. Conclusions

RJ exerted significant antiinvasive activity against very aggressive HCT-116 colorectal cell line by attenuating Snail and vimentin invasive markers, which is significant result of present study, suggesting this natural product as valuable source of anticancer effects. We anticipate that these findings will be focus of increasing attention in both scientific and clinical field of research.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: title; Table S1: title; Video S1: title.

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