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Expression of β-Catenin Marker in Colorectal Cancer Cells after Treatment with Royal Jelly †

Milena M. Jovanović [1,*], Katarina Virijević [2], Dejan Arsenijević [1], Katarina Pecić [3] and Dragana Šeklić [2]

[1] Department of Biology and Ecology, Faculty of Science, University of Kragujevac, 34000 Kragujevac, Serbia
[2] Department of Natural Sciences, Institute for Information Technologies, University of Kragujevac, 34000 Kragujevac, Serbia
[3] Department of Technical and Technological Sciences, Institute for Information Technologies, University of Kragujevac, 34000 Kragujevac, Serbia
* Correspondence: milena.jovanovic@pmf.kg.ac.rs
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Abstract: The deregulation of a Wnt/β-catenin signal pathway is common in colorectal cancer, while β-catenin, its crucial component, is the target for the development of many anticancer therapies. Here, we showed that royal jelly, as a well-known beneficial natural product, can affect β-catenin at both the gene and protein level in HCT-116 colorectal cancer cell line. Our results indicate the effectiveness of royal jelly in targeting crucial markers responsible for the development and progression of cancer. Therefore, royal jelly presents a promising agent for the development of supplementary anticancer therapy.

Keywords: HCT-116 cell line; qRT-PCR; immunofluorescence; nuclear export

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1. Introduction

The Wnt/β-catenin signaling pathway is crucial in a colorectal carcinoma, and its central component β-catenin is the focus of many targeted anticancer therapies [1]. In healthy cells, a Wnt signaling pathway is not active, and β-catenin is mainly located in the peripheral part of the cytoplasm. In the cell membrane area, β-catenin is bound to E-cadherin, thus forming a complex with a role in connecting neighboring cells, which reflects its importance in the epithelium tissue. If it is found free in the cytoplasm, it is immediately degraded by the APC (Adenomatous Polyposis Coli) complex [2]. However, in cancer cells, the Wnt pathway is deregulated and if the APC or β-catenin are mutated or absent, β-catenin is not degraded. It becomes accumulated in the cell cytoplasm and transported into the nucleus, where it acts as a transcription factor regulating cell proliferation, migration, and invasion, and thus the formation of metastases [1].

Data from the literature report the existence of many natural products as sources of biologically active molecules originating from plants and animals with already proven effects on Wnt signaling. The inhibitory properties of these products have been described and are desired when it comes to the Wnt signal pathway. These features designate significant therapeutic potential and make them candidates for successful targeted therapies against cancer [3].

One of recognized natural products reported to be useful as an anticancer agent is royal jelly. This special food derived from bees and intended for their consumption showed inhibitory effects on cancer cell proliferation, tumor growth, and cell invasion [4].

We examined the effect of this natural product, royal jelly (RJ) sampled in Serbia, on β-catenin expression and concentration, since previous studies have shown that RJ has certain suppressive properties on cancer, as well as on its progression in terms of metastasis.

In this study, we examined the effects of RJ on the β-catenin gene and protein expression in colorectal cancer cell line.

2. Methods

β-catenin gene expression was evaluated 24 h after treatment with two RJ concentrations (10 and 100 µg/mL) using the qRT-PCR method, as previously described in detail [5]. Results are presented as the fold change in mRNA expression in a target sample normalized to a reference gene (*β-actin*) and relative to the control sample. Values of relative gene expression were calculated according to $2^{-\Delta\Delta Ct}$ method.

Meanwhile, protein level and localization of β-catenin fractions in the nucleus and cytoplasm were determined by immunofluorescent assay [5] 24 h after treatment of cells with two RJ concentrations (10 and 100 µg/mL). Results of protein expression are presented as relative fluorescence intensity (%).

3. Results

3.1. Gene Expression

The results showed that this natural treatment caused a significant increase in β-catenin gene expression in both applied concentrations (Figure 1). A dose-dependent effect of this treatment compared to the level of the control housekeeping gene *β-actin* can be observed in Figure 1.

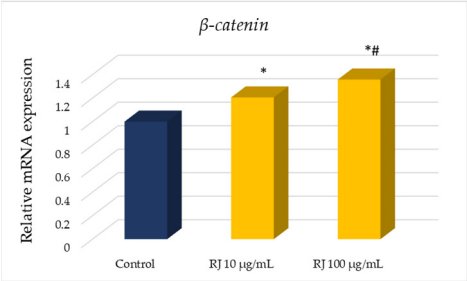

Figure 1. Gene expression of *β-catenin* in untreated (control) and HCT-116 cells treated with RJ after 24 h. * $p < 0.05$ indicates a significant difference compared to control, while # $p < 0.05$ indicates a significant difference between concentrations in treated group.

3.2. Protein Expression and Localization

Our results show that RJ treatment had an impact on total β-catenin concentration when compared to the control values. Namely, both applied RJ concentrations (10 and 100 µg/mL) were able to decrease β-catenin, as it can be observed in Table 1.

Table 1. Level of total β-catenin in HCT-116 cells 24 h after treatment with RJ. Results are presented as relative fluorescence intensity per cell.

Treatment	Value
Control	235,214.6
RJ 10 µg/mL	186,172.9
RJ 100 µg/mL	199,708.6

We noticed a significant decrease of nuclear β-catenin in HCT-116 cells after treatment with RJ (Figure 2). Meanwhile, a dose-dependent increase in the cytoplasmic fraction of this protein was evident after 24 h of treatment with RJ when compared to the control values (Figure 2).

Immunofluorescent technique showed that the localization of nuclear β-catenin was not only limited to the nucleus of control (untreated) HCT-116 cells but was also found in the cell cytoplasm. When these cells were treated with a lower RJ concentration, the localization of this protein was found in both cell compartments again, however, its concentration was lower than in the control cells. Furthermore, a higher RJ concentration induced mostly cytoplasmic localization of this protein (Figure 3a).

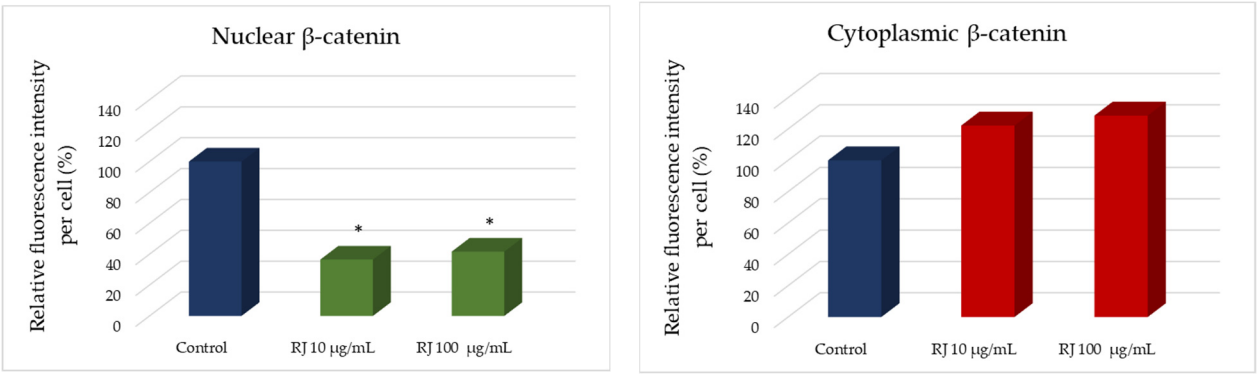

Figure 2. Nuclear and cytoplasmic β-catenin protein expression in control (untreated) and HCT-116 cells treated with RJ after 24 h. * $p < 0.05$ indicates a significant difference compared to control.

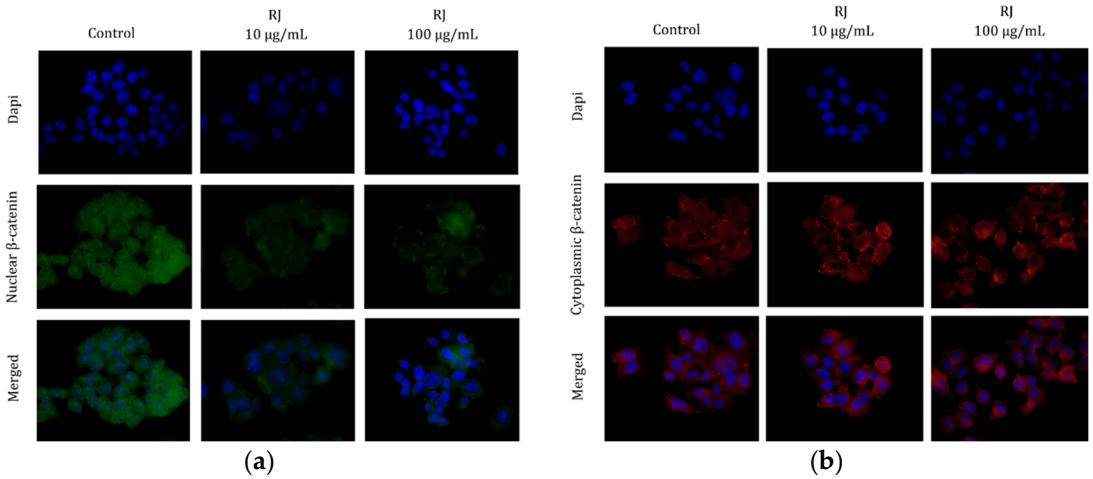

Figure 3. Nuclear (**a**) and cytoplasmic (**b**) β-catenin protein expression and localization in control (untreated) and HCT-116 cells treated with RJ after 24 h.

Meanwhile, we observed the localization of cytoplasmic β-catenin in control and HCT-116 cells treated with RJ and it can be concluded that this protein was mostly concentrated on the cell membrane area (Figure 3b).

4. Discussion

The Wnt/β-catenin signal pathway is conserved signaling and its dysregulation plays a crucial role in cancer development and progression. Hence, agents that are able to target its components are increasingly the focus of worldwide researchers as innovative therapeutic drugs for combating cancer [6].

Considering that royal jelly, as a natural product with proven beneficial effects on human wellness, has already shown significant anticancer activity [4], we aimed to test its potential to modulate the Wnt/β-catenin pathway in selected colorectal cancer cell line.

Firstly, the HCT-116 cell line possesses one mutated *β-catenin* gene allele, while the *APC* gene is a wild type [1,2]. Therefore, this cell line represents a good model system for examining the impact of royal jelly on the central component of Wnt signal pathway, β-catenin.

Our results indicate that the treatment caused the obvious decrease in *β-catenin* gene expression, indicating royal jelly's efficacy in targeting nuclear processes, which is a prominent result that should not be neglected. A previous study conducted by Wang et al. [7] proved that bee products were able to downregulate the *β-catenin* mRNA level in cancer cells, which concords with our results.

Considering total β-catenin protein level in these cells, we observed a decrease of this marker in HCT-116 cells induced by royal jelly. Furthermore, a lower concentration of β-catenin was found in the cell nucleus and a slightly increased level of this protein was found in the cytoplasmic area. This could indicate that treatment started APC-independent signaling pathways are responsible for the export of β-catenin from the nucleus, or that the passive export could occur as result of the treatment effect. This could be a consequence of a reduced interaction of β-catenin with transcription factors and the interaction with DNA in the nucleus. It is known that when present in the cell nucleus, this protein regulates the expression of many markers and plays a significant role in cell proliferation, invasion, and migration. Therefore, it is very important that the level of β-catenin is on a minimum when found in cell nuclei, in order to inhibit cancer development and progression [2].

Furthermore, its relocation from the nucleus to the cytoplasm most probably indicates its migration to the membrane in order to bind to E-cadherin and restore intercellular connections. It is also possible that after its export from the nucleus, royal jelly activated APC protein complexes responsible for the degradation of one portion of β-catenin protein resulted from wild type gene allele that is substrate capable for degradation by APC, therefore its lower concentration in cell cytoplasm.

In conclusion, royal jelly proved to be a powerful agent in affecting *β-catenin* gene expression and in the export of this protein from the nucleus to the cytoplasm where it can be degraded. Similar results in HCT-116 cells treated with natural products have been published earlier by Šeklić et al. [1], confirming the potential that can be found in agents originating from nature regarding targeted activity in cancer cells.

5. Conclusions

Obviously, this natural product was effective in targeting an important component included in a significant signal pathway already deregulated in colorectal cancer cells; however, enormous examinations remain to be completed. We anticipate that these findings will be focus the of increasing attention in both the scientific and clinical fields of research.

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