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Alteration of relative mRNA levels of Tlr-dependent genes in melanoma B16 cells by oligoribonucleotides-D-mannitol treatment

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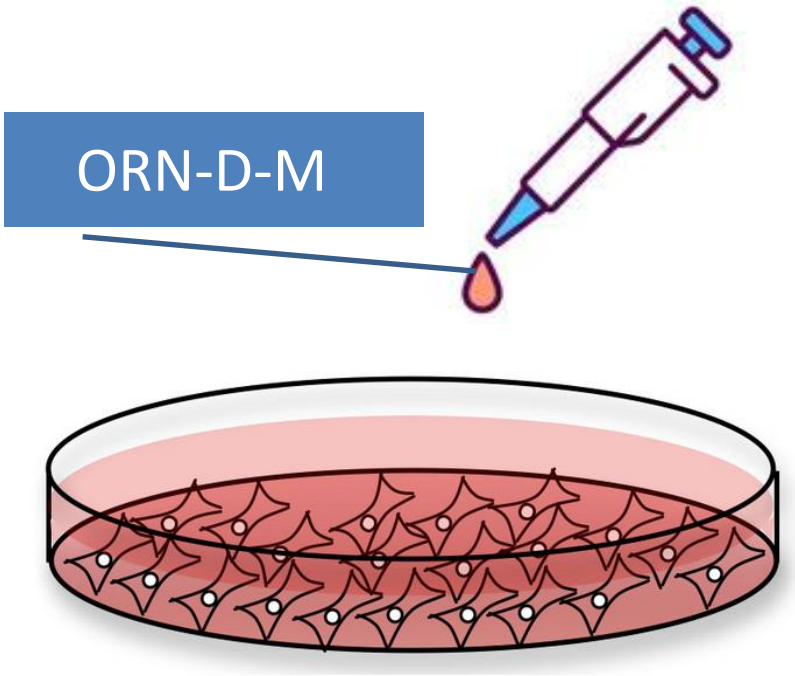
Alteration of relative mRNA levels of Tlr-dependent genes in melanoma B16 cells by oligoribonucleotides-D-mannitol treatment



Murine melanoma B16 culture

- Cell number decrease
- G0/G1 arrest in cell cycle
- Appearance of apoptotic cells
- Increasing of mRNA level of Toll-like receptors and dependent genes

Abstract: Natural oligoribonucleotides in complex with D-mannitol (ORN-D-M) exhibit a range of biological activities, including antiviral and antioxidant effects. In previous investigations, we observed that ORN-D-M demonstrated cytotoxicity on various malignant cell lines in a dose- and time-dependent manner. The aim of the current work was to determine the possible mechanisms of melanoma B16 cell inhibition by ORN-D-M. For this investigation, we examined the relative mRNA expression of various RNA sensors and their downstream-regulated pathways after ORN-D-M treatment of mouse melanoma B16 cells.

It was shown that ORN-D-M caused overexpression of ss-, dsRNA receptors Tlr3, 7, 8, and Eif2ak; the inflammation-suppressive subunit Nfkb1; and IFN type 1. Along with this, downregulation was observed in the mRNA expression of inflammatory cytokines Tnfa and Il1b, which are known as promoters of tumor progression. The ORN-D-M treatment affected apoptosis regulatory molecules, significantly decreasing the relative mRNA level of the antiapoptotic Bcl-2, and slightly increasing proapoptotic Bax.

Therefore, ORN-D-M can be an agonist to Tlr and Eif2ak receptors in melanoma cancer cells, causing the activation of proapoptotic signals through the Nfkb1-dependent pathway.

Keywords: ORN-D-M; RNA receptors; alteration of relative mRNA levels

Introduction

In recent years, many compounds that are contained in natural sources have been identified as biologically active agents. Among them is oligoribonucleotides-D-mannitol (ORN-D-M) – yeast RNA in complex with D-mannitol. This substance has already shown its efficiency against diverse infectious diseases: respiratory infections [1], [2], herpes [3], infectious mononucleosis [4] etc. Besides, ORN-D-M possesses antioxidant and immunomodulative effects [5, 6]. One of the proposed molecular mechanisms for listed biological effects is the triggering of a Toll-like receptor (TLR)-dependent pathway, which activates the pro-inflammatory transcription factor, nuclear factor kappa-B (NF-kB), promoting an innate immune response to viral diseases or oxidative stress. [6, 7].

TLRs are a group of receptors that are directly involved in the regulation of inflammatory reactions and activation of the innate or adaptive immune responses for the elimination of infectious pathogens and cancer debris [8, 9]. These receptors are promising targets for anticancer therapy by the potential in the enhancement of both innate and adaptive immunity, and the induction of apoptosis in TLR-expressing tumor cells [10, 11].

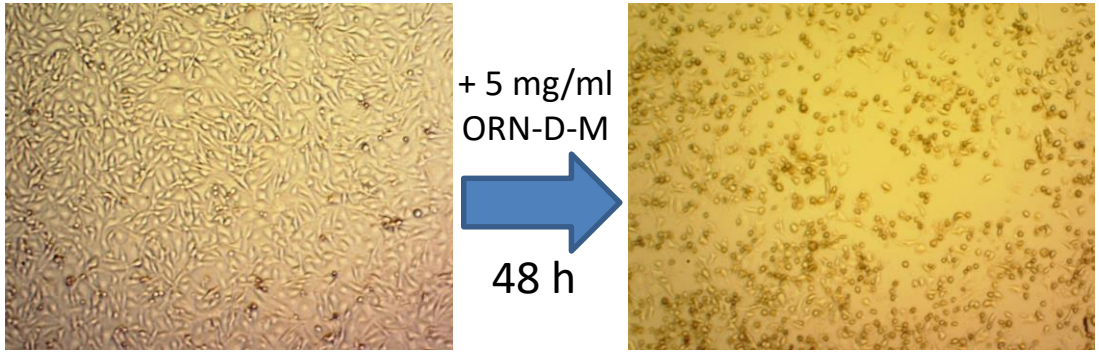


Fig. 1 Microphotographs of murine melanoma B16 culture before and after 48 h treatment with 5 mg/ml ORN-D-M

Previously, we have found, that ORN-D-M inhibits the growth of different cancer cell lines, including murine melanoma B16, in a dose- and time-dependent manner [12], but the mechanism of this activity is still unclear.

Aim

Given the ability of ORN-D-M to act as TLR agonists and induce downstream pathway initiation, which has been demonstrated in animal models, we have investigated the mechanisms and possible involvement of these receptors in the inhibition of B16 cells.

Methods

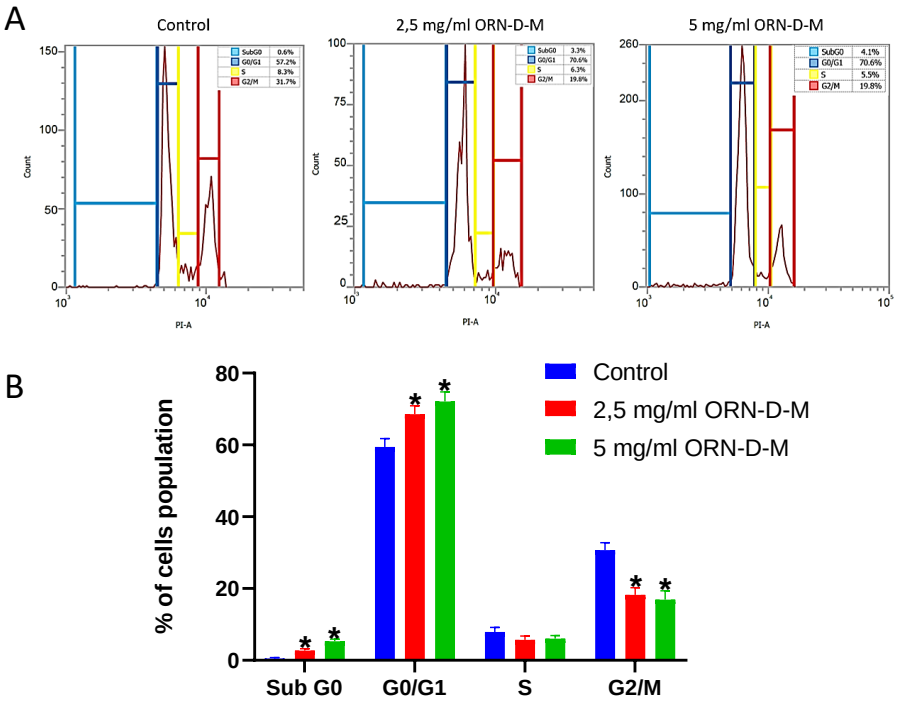
Object: Murine melanoma B16 cell culture

Treatment: 48 hours with ORN-D-M at various concentrations, dissolved in nutrient media.

Subject: Cell cycle and the relative mRNA expression of specific RNA sensors and their pathway components in B16 cells following ORN-D-M treatment.

Methods: Flow cytometry with propidium iodide staining, RT-qPCR.

Results and discussion



ORN-D-M treatment has caused changes in the distribution of B16 cell population in the cell cycle. Specifically, a dose-dependent increase in the number of cells in the G0/G1 phase and a decrease in the G2/M phase were observed, which is characteristic of G0/G1 cell cycle arrest. Additionally, a sub-G0 phase, indicating apoptotic cells, appeared after treatment in both concentration.

Fig. 2 (A) Typical distribution graphics of the population of B16 cells in different phases of the cell cycle during 48-hours treatment with ORN-D-M, obtained using the flow cytometry method with propidium iodide staining.
(B) Distribution of the population of B16 cells in different phases of the cell cycle during treatment with ORN-D-M.
* - p≤0,05 by the Mann-Whitney U test compared to control

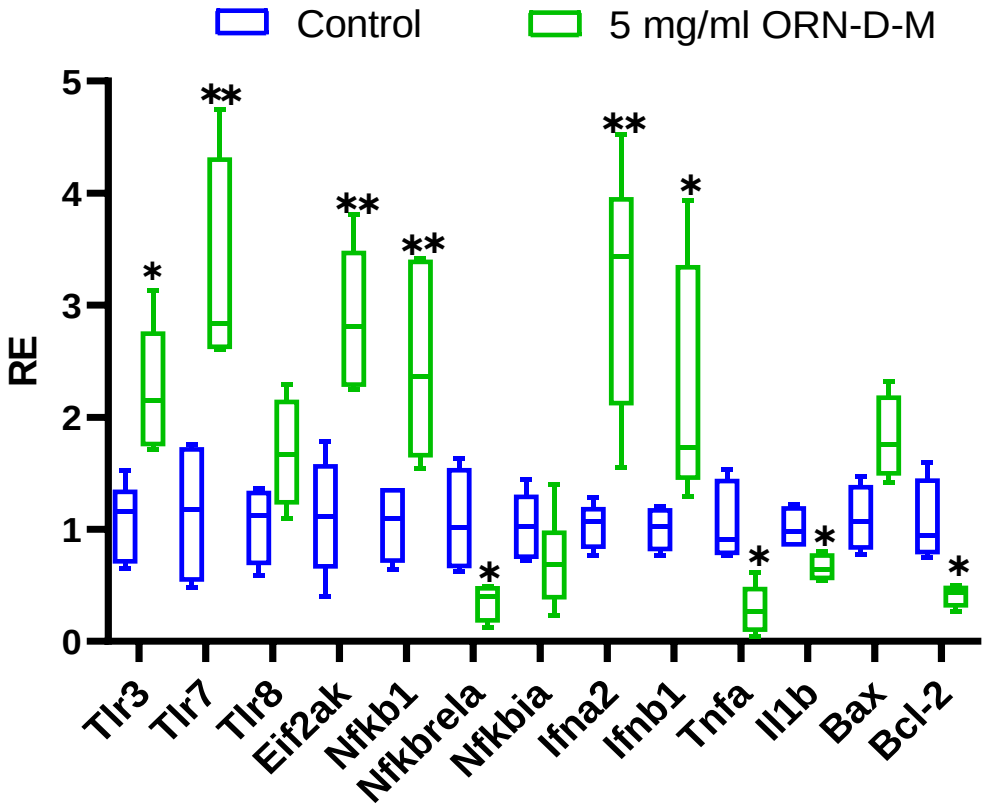


Fig. 3 Relative expression levels of mRNA of different genes in B16 cells in the response to 48-hours treatment with ORN-D-M
* - p≤0,05; ** - p≤0,005 by the Mann-Whitney U test compared to control

Gene	RE experiment/ RE control	P value	Functions of gene product in cell	Literature
Upregulated genes				
Tlr3	2,1	<0,050	Receptors, which indicate pathogen-associated molecular patterns (ss- or ds-RNA) and play a critical role in innate immune responses. They regulate the production of the transcription factor Nfkb and pro-inflammatory cytokines, including interferons type I and Tnfa, Ilb1	[13, 18]
Tlr7	2,6	<0,001		
Tlr8	1,5	0,114		
Eif2ak	2,5	<0,001	ds-RNA binding serine/threonine kinase. It phosphorylates translation initiation factor EIF2S1, which inhibits protein synthesis in the cell	[18]
Nfkb1	2,4	<0,001	Subunit of transcription factor Nfkb, also known as p50. Homodimers of p50:p50 repress gene transcription, dampen inflammatory responses, and abrogate anti-apoptotic signaling. Tlr's target gene	[15]
Ifna2	3,4	<0,001	Cytokines, which regulate immune response. It involved in induction of growth inhibition and mitotic cell cycle perturbation in cancer cells. Nfkb's target gene	[19]
Ifnb1	1,7	<0,050		
Bax	1,8	0,057	Apoptotic activator. It forms a heterodimer with Bcl2, prevents its antiapoptotic signaling	[14]
Downregulated genes				
Nfkbrela	0,4	<0,050	Subunit of transcription factor Nfkb, also known as p65. Dimers of p50:p65 trigger the transcription of inflammatory and cell survival genes. Tlr's target gene	[14]
Tnfa	0,3	<0,050	Inflammatory cytokines, regulated by Nfkb. High levels of these cytokines are connected to tumor progression and survival, and some Tlr's agonists can decrease their production	[17]
Il1b	0,6	<0,050		
Bcl2	0,4	<0,050	Antiapoptotic regulator, which controls the mitochondrial membrane permeability. Nfkb's target gene	[14]
Nfkbia	0,7	0,178	Inhibitor of transcription factor Nfkb. It prevents Nfkb's translocation into the nucleus	[15]

Conclusions

ORN-D-M reduces the proliferation of B16 cells by disrupting the cell cycle, arresting G0/G1 phase and promoting apoptosis.

ORN-D-M triggers mRNA synthesis of Tlr and Eif2ak receptors in murine melanoma B16 cells, likely causing the activation of apoptotic signals through the Nfkb1-dependent pathway.

Literature

1. Andreychin M., Tkachuk Z., Yosyk Y. Efficiency of Nuclex in managing acute respiratory viral infections. *Journal NASU*, 2013. 19;1:100-103.
2. Melnichuk N., Semernikova L. Tkachuk Z. Complexes of Oligoribonucleotides with D-Mannitol Inhibit Hemagglutinin-Glycan Interaction and Suppress Influenza A Virus H1N1 (A/FM/1/47) Infectivity In Vitro. *Pharmaceuticals*, 2017. 10: 71.
3. Tkachuk Z., Rybalko S., Dyadyun S., et al. Antiherpetic activity of the drug Nuclex. *Dopovidi NANU*, 2011. 4: 182-188.
4. Kozko V., Tkachuk Z., Merkulova N., et al. Effectiveness of Nuclex in the therapy of patients with infectious mononucleosis caused by the Estein-Barr virus. ." *Journal NASU*, 2015. 21, 2: 252-257.
5. Marchyshak T., Yakovenko T., Tkachuk Z. Effect of oligoribonucleotides with D-mannitol complexes on oxidative stress indicators against thioacetamide-induced liver fibrosis. *ScienceRise: Biological Science,* 2020. 3, 24: 35-40.
6. Melnichuk N., Kashuba V., Rybalko S. et al. Complexes of Oligoribonucleotides with d-Mannitol Modulate the Innate Immune Response to Influenza A Virus H1N1 (A/FM/1/47) In Vivo. *Pharmaceuticals (Basel),* 2018; 11(3):73
7. Marchyshak T, Yakovenko T, Shmarakov I, Tkachuk Z. The Potential Protective Effect of Oligoribonucleotides-d-Mannitol Complexes against Thioacetamide-Induced Hepatotoxicity in Mice. *Pharmaceuticals (Basel)*. 2018;11(3):77.
8. Sameer A, Nissar S. Toll-Like Receptors (TLRs): Structure, Functions, Signaling, and Role of Their Polymorphisms in Colorectal Cancer Susceptibility. *Biomed Res Int*. 2021. 12;2021:1157023.
9. Kawai T, Akira S. TLR signaling. *Cell Death Differ.* 2006;13(5):16-25.
10. Yang Y, Li H, Fotopoulou C, Cunnea P, Zhao X. Toll-like receptor-targeted anti-tumor therapies: Advances and challenges. *Front Immunol.* 2022. 21;13:1049340.
11. Rolfo C., Giovannetti E., Martinez P. et al. Applications and clinical trial landscape using Toll-like receptor agonists to reduce the toll of cancer. *npj Precis. Onc.* 2023. 7, 26.
12. Kraievska I.M., Tkachuk, Z. Effect of complexes of natural oligoribonucleotides with D-mannitol on the viability of cell cultures of different origin. *Biopolymers and Cell*, 2023. 39:220-230.
13. El-Zayat, S.R., Sibaii, H. & Mannaa, F.A. Toll-like receptors activation, signaling, and targeting: an overview. *Bull Natl Res Cent* 2019. 43, 187.
14. Lawrence T. The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring Harb Perspect Biol.* 2009. 1(6):a001651.
15. Cartwright T, Perkins ND, L Wilson C. NFKB1: a suppressor of inflammation, ageing and cancer. *FEBS J.* 2016;283(10):18, 12-22.
16. Kravtsova-Ivantsiv Y., Shomer I., Cohen-Kaplan V. et al. KPC1-mediated ubiquitination and proteasomal processing of NF-kappaB1 p105 to p50 restricts tumor growth. *Cell*. 2015;161:333–347.
17. Kastenbauer S, Ziegler-Heitbrock HW. NF-kappaB1 (p50) is upregulated in lipopolysaccharide tolerance and can block tumor necrosis factor gene expression. *Infect Immun*. 1999;67(4):1553-9.
18. Reniewicz P, Zyzak J, Siednienko J. The cellular receptors of exogenous RNA. *Postepy Hig Med Dosw* (Online). 2016;70:337-48.
19. Musella M., Manic G., De Maria R. et al. Type-I-interferons in infection and cancer: Unanticipated dynamics with therapeutic implications. *Oncoimmunology*. 2017; 6(5):e1314424.