Original Article

Effects of the Co-Administration of Morphine and Lipopolysaccharide on Toll-Like Receptor-4/Nuclear Factor Kappa β Signaling Pathway of MDA-MB-231 Breast Cancer Cells

Marzieh Kafami[1], Golnaz Vaseghi[2], Shaghayegh Haghjooy Javanmard[2], Manijeh Mahdavi[3], Nasim Dana[2], Nazgol Esmalian-Afyouni[4], Ali Gohari[5]

[1]Cellular and Molecular Research Center, Department of Physiology and Pharmacology, Faculty of Medicine, Sabzevar University of Medical Sciences, Sabzevar, Iran, [2]Applied Physiology Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran, [3]Applied Physiology Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, [4]Applied Physiology Research Center, Cardiovascular Research Center, Isfahan University of Medical Sciences, Isfahan, Iran, [5]Department of Biochemistry and Nutrition, Faculty of Medicine, Sabzevar University of Medical Sciences, Sabzevar, Iran

Abstract

Background: The Toll-like receptor 4 (TLR4) gene promotes migration in adenocarcinoma cells. Morphine is an agonist for TLR4 that has a dual role in cancer development. The promoter or inhibitor role of morphine in cancer progression remains controversial. This study aims to evaluate the effects of morphine on the TLR4, myeloid differentiation primary response protein 88-dependent (MyD88), and nuclear factor-kappa B (NF-κB) expressions in the human MDA-MB-231 breast cancer cell line.

Materials and Methods: The cells were examined after 24 hours of incubation with morphine using the Boyden chamber system. TLR4, MyD88, and NF-κB mRNA expressions were assessed using quantitative real-time polymerase chain reaction (RT-PCR). The concentration of interleukin-2 beta was also measured using the ELISA assay.

Results: According to the findings, three doses of morphine (0.25, 1.25, and 0.025 μM) increased the expression of the TLR4 and NF-κB genes, whereas no significant change was observed in the mRNA expression of MyD88. Furthermore, treatment with morphine and lipopolysaccharide (LPS) significantly decreased the expression of TLR4, MyD88, and NF-κB. However, no significant change was observed in interleukin 2 beta concentration.

Conclusions: These findings confirmed the excitatory effects of morphine on TRL4 expression and the MYD88 signaling pathway *in vitro*.

Keywords: Breast neoplasms, morphine, myeloid differentiation factor 88, Toll-like receptors

Address for correspondence: Dr. Ali Gohari, Assistant Professor of Biochemistry, Department of Biochemistry and Nutrition, Faculty of Medicine, Sabzevar University of Medical Sciences, Tohid Shahr Boulevard, Sabzevar, Iran.
E-mail: gohari.biochem@gmail.com
Dr. Golnaz Vaseghi, Applied Physiology Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran,
E-mail: golnazvaseghi@yahoo.com
Submitted: 07-Apr-2022; **Revised:** 02-Jul-2022; **Accepted:** 27-Jul-2022; **Published:** 28-Jun-2023

Introduction

Breast cancer is the most popular cancer among women worldwide, and it is found to be rising.[1] The prognosis of breast cancer patients depends on the sub-type of breast cancer.[2] Empirical evidence suggests that there is an association between inflammation and carcinogenesis. Furthermore, approximately 15–20% of cancer deaths

Access this article online

Quick Response Code:

Website: www.advbiores.net

DOI: 10.4103/abr.abr_107_22

How to cite this article: Kafami M, Vaseghi G, Haghjooy Javanmard S, Mahdavi M, Dana N, Esmalian-Afyouni N, *et al*. Effects of the co-administration of morphine and lipopolysaccharide on toll-like receptor-4/nuclear factor kappa β signaling pathway of MDA-MB-231 breast cancer cells. Adv Biomed Res 2023;12:149.

are caused by preventable infections.[3] The Breast cancer classification is primarily based on the expression of various immuno-histochemical markers.[2]

Morphine ($C_{17}H_{19}NO_3$) is an exogenous opioid, which is often used as an analgesic drug in cancer patients.[4] Morphine functions through the opioid receptors that are localized in the brain and spinal cord,[5] binding to the opioid receptors on the peripheral tissue and causing complications such as immuno-modulatory, respiratory depression addiction, and tolerance.[6,7]

Morphine and other opioid drugs modulate cellular functions through interactions with both opioid receptors (canonical GPCR opioid receptors; μ, δ, and κ), producing useful effects such as analgesia and other receptors (non-canonical or non-GPCR opioid receptors), for example, Toll-like receptor 4 (TLR4).[8]

Recognizing lipopolysaccharide (LPS), a part of Gram-negative and some Gram-positive bacteria, and triggering a potent immune signaling response are the typical functions of TLR4.[9]

Further studies have shown that various opioid agonists, including both enantiomers of stereo-isomeric opioids such as morphine, methadone, and levorphanol, induce mild but significant activation of TLR4 in the absence of LPS.[10]

Latest studies have reported abnormally upregulated TLR signals in carcinoma during chronic inflammation.[11]

TLR (MyD88-dependent and MyD88-independent) signal pathways through phosphorylation of IκBα, an NF-κB inhibitor, results in ubiquitination and degradation of IκBα, consequently leading to increased NF-κB translocation to the nucleus, where NF-κB finally binds to target promoter regions of genes, to activate transcription and increase tumorigenesis.[8]

Activation of the nuclear factor kappa B (NFκB) is also common in breast cancer and is present in more aggressive tumors because it is associated with resistance to treatment. However, that inhibition can reverse the treatment-resistant phenotype.[12] As a result, new insights into breast cancer treatment identify mechanisms that may block TLR/NFκB activity in breast cancer.[12]

TLR4 is increasingly recognized to play a key role in tumor biology and cancer protection. However, the question of whether TLR4 mediates some of the effects of opioids on tumor growth and metastasis is completely unknown and controversial.[13,14]

Morphine activates TLR4 but suppresses lipopolysaccharide (LPS)-induced TLR4 activation. Overexpression of TLR4 is associated with increased metastasis.[15]

Some study showed that administration of IL-2, along with adoptive transfer of IL-2-cultured anti-tumor T-cells, represents the first effective cancer immuno-therapy in humans and the first curative systemic therapies for solid tumors.[16]

In the past decade, many *in vivo* and *in vitro* studies have demonstrated that morphine could alter tumor growth. Moreover, several experimental studies have shown that morphine could decrease the progression of various human cancer cell lines.[17-19] On the other hand, experiments based on *in vivo* and *in vitro* models have indicated that morphine accelerates cancer cell growth.[20-22]

Consequently, the effects of morphine on cancer growth and metastasis are still debatable.[23] The present study aimed to investigate the effect of morphine on MDA-MB-231, which are epithelial cells isolated from the breast tissue, and the possible mechanisms by which opioids affect tumor growth and metastasis.

Materials and Methods

Cell culture

In the present study, MDA-MB-231 human breast cancer cells were obtained from Pasteur Institute in Tehran, Iran. The cells were grown in 96-well tissue plates and cultured in DMEM 15 medium supplemented with 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 mg/ml). Following that, the cells were incubated in a humidified atmosphere (95% humidity) enriched with 5% CO_2 at a temperature of 37°C. The cell tissues were passaged every 3–4 days. Ethics Committee number: IR.MUI.REC.1394.2.170.

Drug exposure

At this stage, the cells were plated at a density of 3 × 104 cells/ml in 96-well plates and treated for 24 hours by various concentrations of morphine (0.25, 1.25, and 0.025 μM), LPS (1 and 5 μM), and a combination of morphine and LPS.[24] The supernatant was removed and used for the detection of interleukin-2 (IL-2), and the cells were used for assessing gene expression.

RNA extraction and polymerase chain reaction

In total, two micrograms of RNA were treated by DNase I and reverse-transcribed using 100 nanograms of a random hexamer and 1 microliter of SuperScript II reverse transcriptase (Invitrogen Ltd., UK) in accordance with the instructions of the manufacturer. In addition, polymerase chain reaction (PCR) primers were designed using the open-source Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and synthesized using a device obtained from Sigma-Aldrich (Ireland). Table 1 shows the applied primer sequences. Each PCR contained 50 milliliters of a mixture of Taq polymerase (0.5 ml; Invitrogen Ltd., UK) and cDNA (1 ml).

At the next stage, the PCR products were run on 2% agarose gel with a parallel DNA ladder (100 bp; Promega, UK). Real-time PCR (RT-PCR) was also performed using a Light Cycler RNA SYBR Green 1 amplification kit (Roche Applied Science). Measurements were replicated six times, and the acceptable concentration of the total RNA template was determined to be 0.5 μg/ml $^{-1}$. Moreover, the mean cycle

threshold data (Ct) values obtained from the triplicate runs were determined, and quantitative analysis was carried out using the $2^{-\Delta\Delta Ct}$ method. Data calculation was performed using the Light Cycler software version 4.0, and data were normalized based on the levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression.

Cytokine enzyme-linked immuno-sorbent assay

IL-2 level was quantified using a human instant enzyme-linked immuno-sorbent assay (ELISA) kit (eBioscience, San Diego, CA, USA) in accordance with the instructions of the manufacturer. Optical density was also read at 450 nanometers using a VICTORX4 multi-label plate reader (PerkinElmer Life Sciences, Waltham, MA, USA). Standard curves were used to calculate the concentration of IL-2, and the data were expressed as a picogram per milliliter (pg/ml).

Statistical analysis

Data were expressed as the mean and standard error of the mean (SEM). One-sample t-test and one-way analysis of variance (ANOVA) were employed to determine the significance of differences. In all the statistical analyses, a *P* value of less than 0.05 was considered significant.

Results

Effect of morphine on TRL4 expression

To determine whether the effects of morphine are mediated by the activation of the TRL4 receptor, gene expression was evaluated after 24 hours of administrating three doses of morphine. According to the results of RT-PCR, the expression of TLR4 significantly increased at some doses of morphine. However, no significant difference was observed at the morphine dose of 0.025 Mm. TRL4 expression was significantly higher compared to the controls at the dose of 0.25 μM ($P < 0.01$). In addition, the TRL4 receptor increased significantly after treatment with a higher dose of morphine (1.25 μM; $P < 0.05$) [Figure 1a].

Table 1: Primer Sequences Used in RT-PCR

Gene	qPCR primer sequence
TLR4 (TOLL LIKE)	F: 5′:GAGGATGATGCCAGGATGATGTC 3′
	R: 5′:GCCAAGTCTCCACGCAGG 3′
NF-Kβ F	5′:ACATCGTGGTCGGCTTCG 3′
	R: 5′:TGTCATTCGTGCTTCCAGTG 3′
MyD88	F: 5′:TCTCTCCAGGTGCCCATCA 3′
	R: 5′:GGCGAGTCCAGAACCAAGAT 3′

Effect of morphine on NF-κB expression

NF-κB is suspected to be associated with inflammation and carcinogenesis. In this study, we investigated the possible involvement of NF-κB in TLR4 signaling after the morphine treatment. As can be seen in Figure 2a, NF-κB expression significantly increased at all the doses of morphine ($P < 0.05$ and $P < 0.01$).

Effect of morphine on MYD88 expression

To investigate the involvement of the MyD88-dependent pathway, *MYD88* gene expression was assessed, and the obtained results showed the over-expression of *MYD88* in the treatment group compared to the control group. In addition, the lowest dose of morphine (0.025 μM) significantly increased *MYD88* expression ($P < 0.05$) [Figure 3a].

Effect of LPS on TRL4 expression

To find out the possible role of inflammation in the impact of morphine on breast cancer, the expression of TRL4 was evaluated within 24 hours of tumor cell stimulation with LPS and morphine. In response to all the doses of morphine after the LPS treatment, a significant change was observed in the *TRL4* gene expression. As is shown in Figure 1b, pre-treatment with morphine (0.025, 0.25, and 1.25 μM) significantly decreased *TRL4* expression compared to the LPS group ($P < 0.001$) [Figure 1b].

Effect of LPS on NF-κB expression

To investigate whether the expressed TLR was functional following LPS stimulation, we analyzed the expression levels of NF-κB after 24 hours of incubation with LPS and morphine, and a significant decrease was observed in the NF-κB expression in all the study groups as opposed to the LPS group ($P < 0.01$ and $P < 0.001$, respectively) [Figure 2b].

Effect of LPS on MYD88 expression

To recognize the signaling downstream involved in the LPS-induced expression of TLR, we examined the role

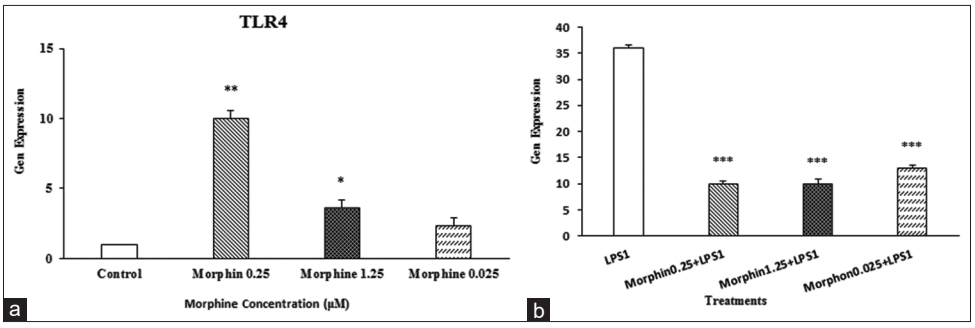

Figure 1: (a) Fold changes of TLR4 expression after incubation with various concentrations of morphine for 24 hours. * * $P < 0.01$ compared with the control. [Mean values (n = 2) are shown]. (b) Fold changes of TLR4 expression after incubation with various concentrations of LPS-induced morphine for 24 hours. * * * $P < 0.001$ compared with the control. Each graph has been represented as mean ± SEM. [Mean values (n = 2) are shown]

of MYD88. After 24 hours of incubation with LPS and morphine, a significant difference was observed between the groups in this regard. As is observed in Figure 3b, *MYD88* gene expression significantly decreased in all the morphine treatments compared to the LPS group ($P < 0.01$).

Effect of morphine on IL2 expression

As is depicted in Figure 4a, no significant difference was denoted between the study groups after cell incubation with various doses of morphine.

Effect of LPS on IL2 expression

As is depicted in Figure 4b, no significant difference was denoted between the study groups after cell incubation with various doses of morphine and LPS.

Discussion

Morphine is a μ-opioid analgesic drug that is used in pain relief in the advanced stages of cancer. Several studies have investigated the effects of morphine on cancer patients, although the exact role of this agent in the regulation of tumor cell growth remains uncertain, which is also true in the case of signaling pathways.[15,24] Several reports have indicated the pro-apoptotic effects of opioids on cancer cells *in vitro*.[25,26] The anti-apoptotic effects of morphine on tumor cells have also been reported.[27] In the present study, the role of TLR4 signaling in the human MDA-MB-231 cell line was evaluated after treatment with morphine, and the obtained results have indicated the up-regulation of TLR4 because of morphine treatment.

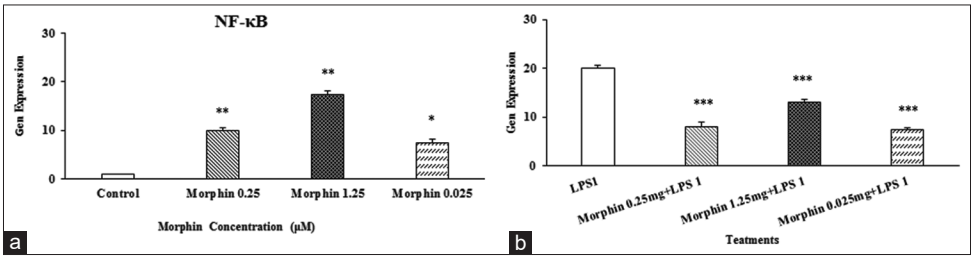

Figure 2: (a) Fold changes of NF-κB expression after incubation with various concentrations of morphine for 24 hours. * $P < 0.05$ and * * $P < 0.01$ compared with the control. Each graph has been represented as mean ± SEM. [Mean values (n = 2) are shown]. (b) Fold changes of NF-κB expression after incubation with various concentrations of LPS-induced morphine for 24 hours. ** $P < 0.01$ and * ** $P < 0.001$ compared with the control. Each graph has been represented as mean ± SEM. [Mean values (n = 2) are shown]

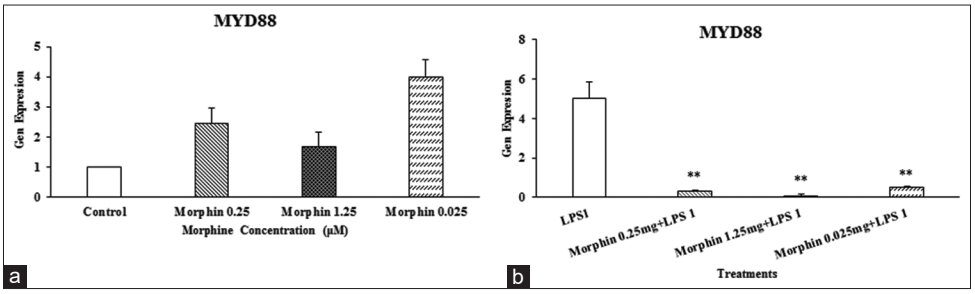

Figure 3: (a) Fold changes of MYD88 expression after incubation with various concentrations of morphine for 24 hours compared with the control. Each graph has been represented as mean ± SEM. [Mean values (n = 2) are shown]. (b) Fold changes of MYD88 expression after incubation with various concentrations of LPS-induced morphine for 24 hours. ** $P < 0.01$ compared with the control. Each graph has been represented as mean ± SEM. [Mean values (n = 2) are shown]

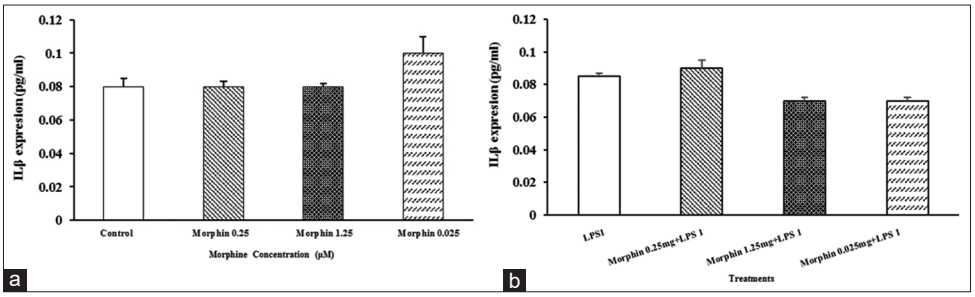

Figure 4: (a) Interleukin 2 concentration after 24 h incubation of cells with various concentrations of morphine compared with the control. Each graph has been represented as mean ± SEM. [Mean values (n = 2) are shown]. (b) Interleukin 2 concentration after 24 h incubation of cells with various concentrations of LPS-induced morphine compared with the control. Each graph has been represented as mean ± SEM. [Mean values (n = 2) are shown]

According to earlier studies, opioids may potentially influence TLR4 signaling.[10,24] In addition to the interaction of opioids with TLR4 in the nervous system, TLR4 signaling seems to influence endothelial cells and immune cells.[28] However, the molecular mechanism of opioid-mediated immune responses has not been adequately clarified.

Early observations have established that NF-κB signaling has involved in opioid-mediated immune responses.[29] NF-κB is a transcription factor. The present study reveals that treatment with morphine increases the NF-κB gene expression. The findings of this study are consistent with another study in this regard, which indicated that treatment with a low dose of morphine could increase NF-kB activation.[30] However, some studies have proposed that morphine could directly block NF-κB through the modulation of nitric oxide release.[31] In the present study, we examined the role of MyD88-dependent TIR4 signaling pathways in acute morphine use. Notably, MyD88 is the adaptor protein of the MyD88-dependent signaling pathway of TLRs, which leads to NF-κB activation.[32,33]

In relation to the current research, the co-treatment of the cells with LPS and morphine led to the potent inhibition of LPS-induced TLR4 activation [Figure 1b]. Despite the agonist effects of morphine on TLR4, *NF-κB*, and *MYD-88* gene expression with the treatment of the cells with morphine alone, inhibitory effects were observed on TLR4 signaling with the co-treatment of the cells with LPS and morphine. This is consistent with a previous study, which indicated that morphine caused the potent inhibition of LPS-induced TLR4 activation.[13] Therefore, it could be inferred that morphine acts as a partial agonist in the presence of LPS.

Activation of TLR4s results in the activation of transcription factors such as NF-κB, which is known to induce the production of pro-inflammatory cytokines. A recent study in this regard demonstrated that the protein expression of IL-2 did not change after morphine treatment. This is inconsistent with the studies showing that the synthesis and secretion of IL-2 inhibited significantly after morphine treatment,[29] although in line with the studies indicating minimal hindrance of IL-2 production after acute morphine treatment IL-2.[34]

Morphine responsiveness is associated with variations in the required dose for patients. Morphine exerts anti-cancer effects at high doses (1–10 mM) by inhibiting the growth of several cancer cell lines *in vitro*.[16] Moreover, the anti-cancer effects of morphine are functionalized through the inhibition of tumor necrosis factor-alpha release, nitric oxide, and reactive oxygen species.[35]

Several research studies reported that high concentrations of morphine induce apoptosis in cancer cells, along with a reduction in Bcl-2 expression, which means that morphine acts through an intrinsic pathway and promotes apoptosis.[36] On the other hand, studies in low doses of morphine have demonstrated that morphine inhibits cell death with the inhibitory impact on the expression of p53 in MDA. MB231 cells or through declining the expression of Bcl-2 in nasopharyngeal carcinoma.[36]

In addition to dose, the duration of morphine treatment is another influential factor in this regard. The reports in this regard are rather inconsistent because of the differences in the studied cell types in experimental studies. A further investigation is required to determine whether morphine treatment inhibits or promotes malignant cell proliferation. In conclusion, our findings confirmed the excitatory effects of morphine on TRL4 expression and MYD88 in the MDA-MB-231 breast cancer cell line signaling pathway. Therefore, morphine may improve cancer tumors and cause metastasis in the patients. However, further investigation is recommended to clearly understand the mechanisms by which morphine affects breast cancer cell metastasis.

Limitations

The limitations of this study were the lack of TNF-α and IFN-β expression in order to evaluate apoptotic pathways using just one cell line and, finally, *in vivo* study has not been performed. Further studies are needed for overcoming these limitations.

Acknowledgements

This research was supported by the Applied Physiology Research Center of Isfahan University of Medical Sciences, Isfahan, Iran.

Financial support and sponsorship

Hereby, we extend our gratitude to Isfahan University of Medical Sciences for the financial support of this research project.

Conflicts of interest

There are no conflicts of interest.

References

1. Dsouza SM, Vyas N, Narayanan P, Parsekar SS, Gore M, Sharan K. A qualitative study on experiences and needs of breast cancer survivors in Karnataka, India. Clin Epidemiol Glob Health 2018;6:69-74.
2. Mehmeti M, Allaoui R, Bergenfelz C, Saal LH, Ethier SP, Johansson ME, *et al*. Expression of functional toll like receptor 4 in estrogen receptor/progesterone receptor-negative breast cancer. Breast Cancer Res 2015;17:1-13.
3. Liu X, Yin L, Shen S, Hou Y. Inflammation and cancer: Paradoxical roles in tumorigenesis and implications in immunotherapies. Genes Dis 2021.
4. Charalambous A, Zorpas M, Cloconi C, Kading Y. Healthcare professionals' perceptions on the use of opioid analgesics for the treatment of cancer-related pain in Cyprus: A mixed-method study. SAGE Open Med 2019;7:2050312119841823.
5. Listos J, Łupina M, Talarek S, Mazur A, Orzelska-Górka J, Kotlińska J. The mechanisms involved in morphine addiction: An overview. Int J Mol Sci 2019;20:4302.
6. Machelska H, Celik MÖ. Advances in achieving opioid analgesia without side effects. Front Pharmacol 2018;9:1388.
7. Odunayo A, Dodam JR, Kerl ME, DeClue AE. State-of-the-art-review: Immunomodulatory effects of opioids. J Vet Emerg Crit Care 2010;20:376-85.
8. Yu H, Lin L, Zhang Z, Zhang H, Hu H. Targeting NF-κB pathway for the therapy of diseases: Mechanism and clinical study. Signal Transduct Target Ther 2020;5:209.
9. AlQranei MS, Senbanjo LT, Aljohani H, Hamza T, Chellaiah MA.

Lipopolysaccharide- TLR-4 axis regulates osteoclastogenesis independent of RANKL/RANK signaling. BMC Immunol 2021;22:23.
10. Zhang P, Yang M, Chen C, Liu L, Wei X, Zeng S. Toll-like receptor 4 (TLR4)/opioid receptor pathway crosstalk and impact on opioid analgesia, immune function, and gastrointestinal motility. Front Immunol 2020;11:1455.
11. Prasad SB, Kumar R. Role of toll-like receptor (TLR)-signaling in cancer progression and treatment. IntechOpen; 2020.
12. Abdin SM, Tolba MF, Zaher DM, Omar HA. Nuclear factor-κB signaling inhibitors revert multidrug-resistance in breast cancer cells. Chem Biol Interact 2021;340:109450.
13. Gabr MM, Saeed I, Miles JA, Ross BP, Shaw PN, Hollmann MW, *et al*. Interaction of opioids with TLR4—mechanisms and ramifications. Cancers 2021;13:5274.
14. Daneshforouz A, Nazemi S, Gholami O, Kafami M, Amin B. The cytotoxicity and apoptotic effects of verbascoside on breast cancer 4T1 cell line. BMC Pharmacol Toxicol 2021;22:72.
15. Vaseghi G, Dana N, Ghasemi A, Abediny R, Laher I, Javanmard SH. Morphine promotes migration and lung metastasis of mouse melanoma cells. Braz J Anesthesiol 2022.
16. Choudhry H, Helmi N, Abdulaal WH, Zeyadi M, Zamzami MA, Wu W, *et al*. Prospects of IL-2 in cancer immunotherapy. BioMed Res Int 2018;2018.
17. Chen Y, Qin Y, Li L, Chen J, Zhang X, Xie Y. Morphine can inhibit the growth of breast cancer MCF-7 cells by arresting the cell cycle and inducing apoptosis. Biol Pharm Bull 2017;40:1686-92.
18. Nishiwada T, Kawaraguchi Y, Uemura K, Kawaguchi M. Morphine inhibits cell viability and growth via suppression of vascular endothelial growth factor in human oral cancer HSC-3 cells. J Anesthesia 2019;33:408-15.
19. Li C, Li L, Qin Y, Jiang Y, Wei Y, Chen J, *et al*. Exogenous morphine inhibits the growth of human gastric tumor in vivo. Ann Transl Med 2020;8:385.
20. Bimonte S, Barbieri A, Cascella M, Rea D, Palma G, Luciano A, *et al*. Naloxone counteracts the promoting tumor growth effects induced by morphine in an animal model of triple-negative breast cancer. *In Vivo* 2019;33:821-5.
21. Zhang J, Yao N, Tian S. Morphine stimulates migration and growth and alleviates the effects of chemo drugs via AMPK-dependent induction of epithelial-mesenchymal transition in esophageal carcinoma cells. Biol Pharm Bull 2020;43:774-81.
22. Cheng S, Guo M, Liu Z, Fu Y, Wu H, Wang C, *et al*. Morphine Promotes the angiogenesis of postoperative recurrent tumors and metastasis of dormant breast cancer cells. Pharmacology 2019;104:276-86.
23. Ghasemi A, Vaseghi G, Hojjatallah A, Haghjooy Javanmard S. The effects of morphine on vascular cell adhesion molecule 1(VCAM-1) concentration in lung cancer cells. Arch Physiol Biochem 2021:1-5.
24. Haghjooy-Javanmard S, Ghasemi A, Laher I, Zarrin B, Dana N, Vaseghi G. Influence of morphine on TLR4/NF-kB signaling pathway of MCF-7 cells. Bratisl Lek Listy 2018;119:229-33.
25. Theile D, Mikus G. Methadone against cancer: Lost in translation. Int J Cancer 2018;143:1840-8.
26. Yin D, Woodruff M, Zhang Y, Whaley S, Miao J, Ferslew K, *et al*. Morphine promotes Jurkat cell apoptosis through pro-apoptotic FADD/P53 and anti-apoptotic PI3K/Akt/NF-κB pathways. J Neuroimmunol 2006;174:101-7.
27. Zhang XY, Liang YX, Yan Y, Dai Z, Chu HC. Morphine: Double-faced roles in the regulation of tumor development. Clin Transl Oncol 2018;20:808-14.
28. Tariq I, Farhan A, Ashfaq UA, Qasim M, Rehman M, Humayun F, *et al*. Study on structural insight of the analysis of negative effects of opioids analgesics in naltrexone with TLR4 Mutations. Pak J Pharm Sci 2019;32(Suppl 1):345-51.
29. Eisenstein TK. The Role of Opioid Receptors in Immune System Function. Front Immunol 2019;10:2904.
30. Chaves C, Remiao F, Cisternino S, Decleves X. Opioids and the blood-brain barrier: A dynamic interaction with consequences on drug disposition in brain. Curr Neuropharmacol 2017;15:1156-73.
31. Rodriguez M, Lapierre J, Ojha CR, Estrada-Bueno H, Dever SM, Gewirtz DA, *et al*. Importance of autophagy in mediating human immunodeficiency virus (HIV) and morphine-induced metabolic dysfunction and inflammation in human astrocytes. Viruses 2017;9:201.
32. Zheng C, Chen J, Chu F, Zhu J, Jin T. Inflammatory role of TLR-MyD88 signaling in multiple sclerosis. Front Mol Neurosci 2020;12:314.
33. El-Zayat SR, Sibaii H, Mannaa FA. Toll-like receptors activation, signaling, and targeting: An overview. Bull Natl Res Centre 2019;43:187.
34. Chen S-H, Chen S-S, Wang Y-P, Chen L-K. Effects of systemic and neuraxial morphine on the immune system. Medicine 2019;98:e15375.
35. Tuerxun H, Cui J. The dual effect of morphine on tumor development. Clin Transl Oncol 2019;21:695-701.
36. Rezaeigazik M, Nabiuni M, Jalali H, Kabuli M. Investigating the effects of morphine on survival and sensitivity to cisplatin in ovarian cancer cells. J Cell Mol Res 2019;11:1-7.