Research Article

Effect of Nanocurcumin in Combination with Methotrexate on Telomerase Activity, NF-kb Expression, and Proliferation Index of Bewo Choriocarcinoma Cells

Pengaruh Kombinasi Nanokurkumin dengan Methotrexate terhadap Aktivitas Telomerase, Ekspresi NF-kb, dan Indeks Proliferasi Sel Koriokarsinoma Bewo

Subandi[1], Sigit Purbadi[2]

[1]Master Program in Midwifery
Departement of Midwifery
Faculty of Medicine Universitas Brawijaya
Departement of Obstetrics and Gynecology
Universitas Brawijaya Hospital
Malang
[2]Departement of Obstetrics and Gynecology
Faculty of Medicine Universitas Indonesia
Dr. Cipto Mangunkusumo General Hospital
Jakarta

Abstract

Objectives: To determine the anti-cancer effect of nanocurcumin on choriocarcinoma.

Methods: This study observes the telomerase activity, NF-κB expression, and BrdU proliferation index in cultures of BeWo choriocarcinoma cell line (ATCC CCL-98) exposed to MTX and nanocurcumin in various doses. The sample used in this study consisted of 4 groups of BeWo cells that received a combination of MTX and nanocurcumin, and 2 other groups for positive and negative control.

Results: There was a decrease in telomerase activity, NF-κB expression, and BrdU proliferation index in the 4 treatment groups compared to the negative and positive control group ($p \leq 0.05$).

Conclusion: Nanocurcumin and MTX decrease telomerase expression, NF-κB expression, and the BrdU proliferation index in choriocarcinoma BeWo cell line culture faster than MTX alone.

Keywords: BrdU index proliferation, choriocarcinoma, methotrexate, nanocurcumin, NF-κB expression, telomerase expression.

Abstrak

Tujuan: *Untuk mengetahui pengaruh anti kanker nanokurkumin pada koriokarsinoma.*

Metode: *Penelitian ini mengamati aktivitas telomerase, ekspresi NF-kB, dan indeks proliferasi BrdU pada kultur sel koriokarsinoma BeWo (ATCC CCL-98) yang dipapar MTX dan nanokurkumin dalam berbagai dosis. Sampel yang digunakan dalam penelitian ini terdiri dari 4 kelompok sel BeWo yang mendapat kombinasi dari MTX dan nanokurkumin, dan 2 kelompok lainnya untuk kontrol positif dan negatif.*

Hasil: *Terdapat peningkatan dalam aktivitas telomerase, ekspresi NF-kB, dan proliferasi BrdU pada empat kelompok perlakuan yang dibandingkan dengan kelompok kontrol positif dan negatif ($p<0.05$).*

Kesimpulan: *Nanokurkumin dan MTX meningkatkan aktivitas telomerase, ekspresi NF-kB, dan indeks proliferasi BrdU dalam kultur sel koriokarsinoma BeWo yang lebih cepat daripada MTX sendiri.*

Kata kunci: *Aktifitas telomerase, ekspresi NF-kB, koriocarsinoma, Metotreksat, Nanokurkumin, Proliferasi indeks BrdU.*

Correspondence author. Subandi. Department of Obstetrics and Gynecology.
Faculty of Medicine Universitas Brawijaya. Universitas Brawijaya Hospital. Malang.
Email: desobg@ub.ac.id

Received: April, 2022 Accepted: March, 2023 Published: April, 2023

INTRODUCTION

The incidence rate of gestational trophoblastic disease is 11.5 per 1,000 pregnancies in Indonesia. This has made Indonesia as one of the highest incidence rates of gestational trophoblastic disease in the world. Around 15-20% of patients with complete hydatidiform mole will experience malignant transformation into a malignant trophoblastic disease.[1,2]

Fifty percent of all gestational trophoblastic disease presented as hydatidiform mole, 25% cases are associated with abortion or ectopic pregnancies, and 25% occurred after term and preterm deliveries. Choriocarcinoma is the malignant form of the gestational trophoblastic diseases. Around 2.5% of complete mole cases advance into choriocarcinoma.[3,4] Choriocarcinomas can be distinguished into gestational and non-gestational choriocarcinoma. Gestational choriocarcinoma occurs mostly in reproductive women, usually 1 year after molar or non-molar pregnancy. Non-gestational choriocarcinoma originates from germ cells or trophoblast differentiation in endometrial carcinoma.[2]

Choriocarcinoma is the most common malignant trophoblastic disease compared to an invasive mole or placental site trophoblastic tumor (PSTT). Malignant trophoblastic disease is a malignancy with high levels of invasion and metastasis, in which invasion can alter the myometrium and cause uterine perforation. Distant organ metastases occur in about 19% of choriocarcinomas. The metastases occur hematogenously, most often to the lungs (80%), the vagina (30%), the brain (10%), the liver (10%), the kidneys, and gastrointestinal tract. However, choriocarcinoma is responsive to chemotherapy and its cure rate is 90%. One of the chemotherapy agents used to treat choriocarcinoma is methotrexate (MTX). The mechanism of MTX in choriocarcinoma sensitivity could be associated with the inhibition of RAD51 expression due to DNA damage and the suppressed homologous recombination in tumor cells induced by MTX.[5,6] Methotrexate itself comes with a variety of toxicities. The current development of research on natural drugs, such as curcumin is needed to reduce the toxicity caused by methotrexate and improve the effects of chemotherapy-resistant cases. In addition, the incidence of recurrence in post-chemotherapy patients with choriocarcinoma is about 27% in high risk cases.[1,2]

Human Telomerase Reverse Transcriptase (commonly referred to as hTERT) in humans, is a catalytic subunit of the enzymes telomerase that is associated with repetition of DNA sequences at the end of the eukaryotic chromosomes that work to prolong telomeres. The expression of hTERT is generally required for the development and proliferation of cancer cells.[7] Expression of hTERT has an important role in the continuity of the malignant treatment process. Activation of this enzyme often occurs simultaneously with malignancy. Telomerase expression can be found in choriocarcinoma and exhibits an active and strong properties in the pathogenesis of choriocarcinoma.[8]

NF-κB has physiological function as the main transcription factor for the regulation of immune response and inflammation on various tissue. NF-κB-induced gene expression plays an important role in the tumor and metastasis, where NF-κB-dependent transcription generates the protein which causes cells' resistance to apoptosis, stimulates oncogenic activity, angiogenesis and also stimulates the cells' proliferation.[9,10]

Curcumin (*Dipheruloylmethane*) [*1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5dione*] is a natural phytochemical that was considered has chemopreventive and chemotherapeutic activity by working on 'multiple signaling pathways', including cell survival regulation, proliferation, and angiogenesis process in cancer cells, without any toxic effect on healthy cells.[11] Some literatures have reported some biological aspects of curcumin. The biological aspect of curcumin that is currently being developed is its pharmacological properties such as anti inflammation, antihepatotoxic, and anti-cancer. Curcumin, as an anticancer, is associated with its activity as antiradical, antioxidant, antiproliferation, antiinflammation, and antiapoptotic compound. That mechanism goes by inhibiting hTERT activity that plays a role in cell proliferation, so it decreases BrdU index proliferation[12] and inhibits cell growth, stimulating apoptosis by suppressing NF-κB transcription activity. However, the curcumin in its oral preparation has a low bioavailability, hence the analog structure that has the feature of faster absorption and longer half-life is preferred. Oral administration of curcumin nanoparticles improves the bioavailability up to 5,6 times and longer half-life compared to its original form.[13]

Eventhough curcumin is considered as a novel chemotherapeutic agent, little is known

about its effect in choriocarcinoma. This study aims to investigate the effects of nanocurcumin in addition to methotrexate toward telomerase activity, NF-κB expression, and BrdU proliferation index in BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98), compared with methotrexate only.

METHODS

This study is an experimental study, conducted in vitro using BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98) that is intervented in selected doses of nanocurcumin 25,50,100 µg/mL and MTX 100 µg/mL and in telomerase activity, NF-κB expression and BrdU proliferation index were assessed. This study was held in cell/tissue culture laboratory, Faculty of Medicine, Universitas Brawijaya, Malang, from March to April 2018. The sample was taken from BeWo Choriocarcinoma from American Type Culture Collection CCL-98.

A preliminary study was done to determine the dosage of MTX and the result was 100µg/ml, which showed MTX suppresses the NF-κB expression faster compared to the other dosages (999.22±181.39 density/mm^2, p≤0.001). Repetition was done for 5-plo in different dosages. Five steps are 5 samples consist of BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98) without curcumin nor MTX, 5 samples consist of BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98) received MTX 100 µg/ml, 5 samples consist of BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98) received MTX 100 µg/ml + Nanocurcumin 25µg/ml, 5 samples consist of BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98) received MTX 100 µg/ml + Nanocurcumin 50µg/ml, 5 samples consist of BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98) received MTX 100 µg/ml + Nanocurcumin 100µg/ml, 5 samples consist of BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98) received MTX 100 µg/ml + Nanocurcumin 200µg/ml.

After receiving the intervention, BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98) were incubated for 6 hours. Each sample underwent 5 times of replication before being assessed. Telomerase activity was measured by a Real-Time PCR. A mixed component that was measured consisted of 12.5 µl *Premix* QTD 2x, 1.0 µl of cell or tissue, 11.5 µl of qualified PCR water, with the total volume of 25.0 µl. Upon adding 1 µl of the examined preparations, RT PCR detection program was arranged into 5 steps among others: telomerase action, PCR initial, 3-steps cycling, annealing, and extension. PCR quantification was shown on the data that was collected on screen during the PCR process in a real-time manner, with the cycle threshold or CT value after cycles are done.

NF-κβ was measured by immunohistochemistry and BrdU was measured by flow cytometry. The observation was made on each slide examining how many cells expressed NF-κB with immunohistochemistry staining, which was counted per 200 cells, captured with 400x magnification microscope, and Adobe Photoshop CS2 program to assess the NF-κβ immunohistochemistry thickness on the nucleus with RGBbv unit. Bromodeoxyuridine marking was equipped in the *in vitro* BrdU marking.

Data analysis was done using number of tests consecutively: Shapiro-Wilk test for normality test in data with ratio scale, Independent T-test to compare 2 groups of sample, One Way Anova Test to compare more than 2 groups of sample, and Dunnet test for multiple comparisons if the result from One Way Anova Test show significant differences and disapproval of H_0 (p-value ≤0,05). The data were analyzed using *Statistical Package for the Social Sciences* (SPSS) For Windows Version 23.

This study was reviewed and approved by the Institutional Review Board and Ethical Committee Dr. Cipto Mangunkusumo, a national reference and teaching hospital. The samples were maintained under applicable medical ethical standards.

RESULTS

Nanocurcumin effects on Telomerase Expression

This study showed the decreasing mean value of telomerase expression between the control group (-) (75.50±4.11%) and the control group (+) (68.22±3.34%) as depicted in Figure 1. The highest mean value of telomerase expression was found in the negative control group (no intervention), and followed by positive control group (received MTX 100 µg/mL), and the number was decreasing in the following group as the nanocurcumin received was increasing. Thus, the administration of nanocurcumin + MTX 100 µg/mL was able to decrease telomerase expression faster than only

MTX 100 µg in BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98).

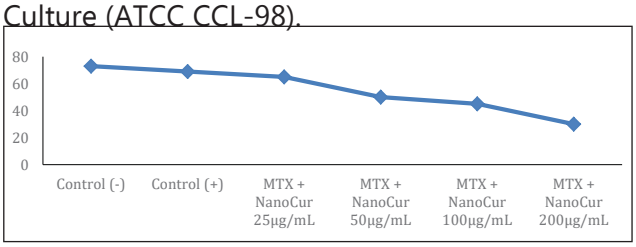

Figure 1. The mean value of Telomerase expression.
*p-value ≤ 0.05 versus negative control; *p-value ≤ 0.05 versus positive control.

Effect of Nanocurcumin on NF-κB Expression

This study showed the decreasing mean value of NF-κB in the culture of *cell line* choriocarcinoma *BeWo* (*ATCC CCL-98*) between the control group (-) (2455.27±124.12 density/mm^2) and control (+) (2065.29±264.02 density/mm^2) as depicted in Figure 2. The highest mean value of telomerase expression was found in the negative control group (no intervention) and followed by positive control group (received MTX 100), and the number decreased in the following group as the nanocurcumin received was increasing. Thus, the administration of nanocurcumin + MTX 100 µg were able to decrease NF-κB expression faster than only MTX 100 µg in BeWo Choriocarcinoma Cell Line Culture ATCC CCL-98).

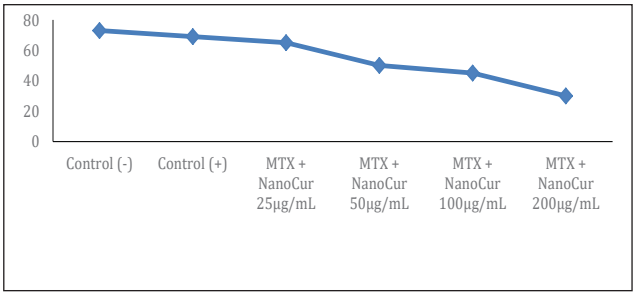

Figure 2. The mean value of NF-κB expression

*p-value ≤ 0.05 versus negative control; *p-value ≤ 0.05 versus positive control.

Effect of Nanocucumin on BrdU Proliferation Index

This study showed the decreasing mean value of proliferation of BrdU in the culture *cell line* choriocarcinoma *BeWo* (*ATCC CCL-98*) between the control group (-) (7.26±0.96%) with the control group (+) (5.81±0.72%) as depicted in Figure 3. The highest mean value of BrdU Proliferation index was found in the negative control group (no intervention) and followed by positive control group (received MTX 100), and the number was decreasing in the following group as the nanocurcumin received was increasing. Thus, the administration of nanocurcumin + MTX 100 µg was able to decrease BrdU proliferation index faster than only MTX 100 µg in BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98).

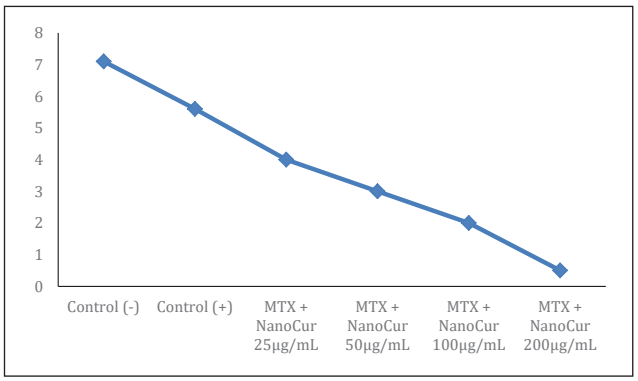

Figure 3. The mean value of BrdU proliferation index
*p-value ≤ 0.05 versus negative control; *p-value ≤ 0.05 versus positive control.

DISCUSSION

In this study, a preliminary study was conducted to determine the optimum dose of MTX that would be used for the intervention on BeWo choriocarcinoma cell line culture. The subject of the preliminary study received several different doses of MTX. In the observation group, MTX was given in low to higher doses. The study showed a 100 µg/mL MTX suppressed the NF-κB expression faster than that of other doses (999.22±181.39 density/mm2, p ≤ 0.001). These results are supported by previous studies showing the administration of medium-dose MTX 10 µg/mL - 100 µg/mL in cancer cells after an 8-hour incubation that could induce apoptosis through decreased cell viability.[14] Methotrexate activates the formation of polyglutamate, AICAR inhibitor, which increases extracellular adenosine secretion. Medium dose methotrexate induces apoptosis of dependent T cells via CD95 dependent pathway.[15] High dose methotrexate (> 100 µg/mL) suppresses the proliferation process, but it did not induce apoptosis. Suppression of proliferation through RNA and DNA barriers by inhibition of dihydrofolate reductase in phase cell cycle S. The suppression of proliferation occurs due to number of suppressors of inflammatory factors such as IL-1β, IL-6, TNF α, COX 2, and LOX.[1]

This study showed a decrease in telomerase expression by administering MTX on BeWo choriocarcinoma cell line culture. Previous

research has shown that methotrexate caused DNA replication in S phase to be halted so that no changes in telomerase expression occurred.[16] Inhibition of cell proliferation is a result of the cytotoxic effect of methotrexate by inhibition of dihydrofolate reductase (DHFR). This inhibition makes tetrahydrofolate depletion, resulting in inhibition of purine and pyrimidine synthesis precursors. Thymidylate can not catalyze dUMP methylation into dTMP without THF, resulting in *thymineless death* and inhibition of purine synthesis. Inhibition of pyrimidine synthesis precursors occurred because methotrexate also directly inhibits AICAR (ATIC) transfomilase and EAR (EART) transfomilase.[17] This results in chromosome instability so that the synthesis of hTR and hTERT at the onset of S phase is inhibited. The suppression of hTERT expression causes the downregulation of C-myc resulting in cell proliferation not occurring.[18–20] Other studies have shown that methotrexate could decrease the metabolism of folate and inhibited hTERT expression in cancer cells. Methotrexate inhibits methionine thereby decreasing S-adenosyl methionine, a methyl donor in protein methylation of folate metabolism. In addition, the decrease in folate metabolism is also due to decreased activation of ERK and AKT signal pathways that function as a proliferation factor.[17]

The results of this study showed that the administration of MTX + nanocurcumin decreased telomerase expression in BeWo choriocarcinoma cell line culture with the average of telomerase expression was smaller than that of the positive control group. This is in accordance with other studies showing that telomerase expression decreased after nanocurcumin administration for 48 hours. Downregulation of telomerase expression is attributed to the suppression of hTERT expression or local suppression of nucleotide translocation on independent hTERT cells.[21] This result is supported by a study by Shariati et al indicating that there was a change in telomerase expression after a low dose of nanocurcumin for 72 hours.[22] The decrease of telomerase expression is due to the accumulation of nanocurcumin inside the cell, leading to the increase of ROS accumulation, increasing of Smad3 and E2F1 causing a decrease in expression hTERT.[23]

This study showed that a combination of MTX + nanocurcumin had a synergistic effect on telomerase expression. Both drugs together decreased hTERT expression directly and by increasing oxidative stress in the cell, so telomerase expression got lower.

NF-κB transcription factor is suspected of having a role in the development of cancer caused by oncogenic mutations in some malignancies. NF-κB acts as a link between inflammation and cancer. The expression of NF-κB is the result of inflammation or the effect of the formation of an inflammatory microenvironment in the development of malignancy.

The results of this study showed a decrease in expression of NF-κB after MTX administration on BeWo choriocarcinoma cell line culture. Inhibition of expression NF-κB has a role in the proliferation process through multiple pathways. Methotrexate inhibits DHFR through two inflammatory pathways: BH2 inhibition that decreases BH4 expression, thus uncoupling the iNOS enzyme, increasing the production of ROS and activating JNK and the release of dependent adenosine, AICAR, activating of adenosine receptors. Both of these pathways results in a decrease in the production of NF-κB.[24] These findings are being reinforced by previous studies showing that methotrexate inhibited NADPH dehydrogenase dependent which are2-oxoglutarate and pyruvate dehydrogenase. This resulted in a decrease of glutathione, acytoplasmic antioxidants. In addition, ROS accumulation resulted in the disruption of potential mitochondrial membranes through activation of the JNK kinase. JNK kinase induced the proapoptotic target genes and activates apoptosis.[17] Another pathway that suppresses TNF is through the release of adenosine and its interaction with adenosine of the A3 receptor as well as the inhibition of pyrimidine synthesis. Methotrexate inhibits genes regulated by NF-κB ie COX-2, iNOS, MMP-9, IL-1, IL-2, IL-6, and GM-CSF.[25]

This study also showed that there was a decrease in NF-κB expression by giving MTX +nanocurcumin to BeWo coriocarcinoma cell line culture. Curcumin is a polyphenol that suppresses various anti-inflammation such as TNFα, IL-1, IL-2, IL-6, IL-8, and IL-12. Inactivation of TNF α led to inhibition of IKK activation, inhibition of IkB α phosphorylation, and translocation of p65 subunit from NF-κB.[26] Increased NF-κB expression was influenced by activation of protein kinase and protein C. In this study, administration of nanocurcumin resulted in ROS accumulation in cells which destroy TNF and H2O2. Both processes result in the inhibition of protein kinase.[27]

This study also showed that the administration of MTX + nanocurcumin greatly decreased NF-κB expression in BeWo coriocarcinoma cell line culture. MTX and curcumin inhibit NF-κB expression in the same pathway through inhibition of IkBα phosphorylation. In addition, both of these regimens increase ROS levels in the nucleus activating cytochrome C that induces apoptosis. This results in the simultaneous administration of MTX and nanocurcumin further decreasing the expression of NF-κB. Curcumin increases MTX and folate acid in KG-1 cells by increasing the sensitivity of B-mRNA and protein folate receptors. In addition to that, curcumin increases the cytotoxic effect of MTX. Combination of drugs (curcumin + doxorubicin) with nanoparticle formula inhibits drug resistance logging by inhibiting Bcl-2 expression and prolonging doxorubicin effect in cells.[28]

BrdU is a thymidine analog that joins a single-stranded DNA in a cell cycle. BrdU is used as a proliferation marker and in cell migration that is inserted in the S-phase cell cycle and persists in the cell.[29]

This study showed the decrease of BrdU index proliferation by the administration of MTX on BeWo choiocarcinoma cell line culture. Methotrexate interferes with the kinetic of cell cycle by discontinuing it on S-phase. Methotrexate inhibits dihydrofolate reductase, altering neutrophil formation in the hydrolysis of dUMP to dTMP in pyrimidine synthesis. In S-phase, p53 destroys thymidine and stranded DNA by DNA helicase. The disruption of synthesis of purines cause the "thymineless stress", an apoptosis process.[16,17]

The results of this study showed that the administration of MTX + nanocurcumin decreased the proliferation index in of BeWo choriocarcinoma cell culture with mean value of BrdU proliferation index smaller compared to the mean value of positive control group. The administration of nanocurcumin causes the accumulation of ROS in cells and the downregulation of apoptotic suppressor proteins (BcL-2 and BcL-xL). The inreased ROS also causes downregulation of the API-1 binding factor, resulting in the G0 / G1 phase stalled. Nanocurcumin makes the cell cycle stop on G1 / S phase by inhibiting the binding of Cdk 4 and Cdk 6, which are G1 / S regulators. Cyclin D1 is inhibited through the Ras / ERK pathway and activation of STAT3.[30]

This study also showed that the administration of MTX + nanocurcumin decreased the BrdU proliferation index greater compared to MTX only in cell culture line of BeWo coriocarcinoma. The administration of nanocurcumin decreases the BrdU proliferation index in various cell cycle phases that have a synergistic effect with methotrexate administration. From these findings, this study showed that the administration of nanocurcumin + MTX could give a better outcome in patients with choriocarcinoma compared with MTX administration alone. The decrease of NF-κB expression could stimulate the apoptosis which its index is used as a prognostic factor in gestational trophoblastic disease.

The limitation of this study is the use of relatively small sample for each group. This study was conducted in vitro using BeWo Choriocarcinoma Cell Line Culture (ATCC CCL-98). However, further study is needed, in primary culture and animal study, to evaluate the correlation of telomerase expression, NF-κB expression with decreasing BrdU proliferation index on nanocurcumin + MTX administration therefore the biomolecular patophysiology could be understood clearly.

CONCLUSIONS

The administration of nanocurcumin + MTX has an effect on decreasing telomerase expression, decreasing NF-κB expression, and decreasing BrdU proliferation index on BeWo coriocarcinoma cell line culture (ATCC CCL-98); and the administration of nanocurcumin + MTX further decreased the expression of NF-κB and further lowered the BrdU proliferation index on BeWo coriocarcinoma cell line culture (ATCC CCL-98) compared with MTX.

Data Availability

The data used to support the findings of this study are available on request.

CONFLICT of INTEREST

The authors declare no conflict of interest.

FUNDING STATEMENT

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

1. Bratakoesoema D. Penyakit Trofoblas Gestasional. In: Aziz M, Andrijono, Saifuddin A, editors. Buku Acuan Nasional Onkologi. 1st ed. Jakarta: Yayasan Bina Pustaka Sarwono Prawirohardjo; 2006: 532–68.
2. Hextan Y.S. Ngan, Michael J. Seckl, Ross. S. Berkowitz, et al,. Diagnosis and Management of GestationalTrophoblastic Disease. Int J Gynecol Obstet. 2021. 155 Suppl 1(Suppl 1):86-93 https://doi.org/10.1002/ijgo.13877
3. Braga A, Mora P, Melo AC de, et al. Challenges in the diagnosis and treatment of gestational trophoblastic neoplasia worldwide. World J Clin Oncol. 2019 ;10(2):28–37.doi.org/10.5306/wjco.v10.i2.28
4. Jagtap SV. Aher V, Gadhiya S, et al,. Gestational Trophoblastic Disease Clinicopathological Study at Tertiary Care Hospital. J Clin Diagnos Res. 2017;11(8):EC27–EC30 https://doi.org/10.7860/JCDR/2017/27232.10458
5. I Nyoman B. Mahendra, Bagus N. Brahmantara, and William A. Setiawan. A Review of Current Management of Gestational Trophoblastic Disease. Eur J Med Health Sci. 2022;4(5):7-10. doi:10.24018/ejmed.2022.4.5.1403
6. Xie L, Zhao T, Cai J, Su Y, Wang Z, Dong W. Methotrexate induces DNA damage and inhibits homologous recombination repair in choriocarcinoma cells. Onco Targets Ther. 2016 ;9:7115–22. https://doi.org/10.2147/OTT.S116387.
7. Marta Dratwa, Barbara Wysoczan´ska, Piotr Łacina, et al,. TERT Regulation and Roles in Cancer Formation. Front Immunol. 2020; 11:589929. doi: 10.3389/fimmu.2020.589929 .
8. Nurseta T, Wijasa A, Sitompul B. Impact of Ethinyl Estradiol to Human Telomerase Reverse Transcriptase Activity on Complete Hydatidiform Mole Culture. Inajog. 2016;4(2):93-6. https://doi.org/10.32771/inajog.v4i2.82
9. Kresno SB. Ilmu Dasar Onkologi. Second Edi. Jakarta: Badan Penerbit Fakultas Kedokteran Universitas Indonesia. 2011: 66–129.
10. Sudiana I. Teknologi ilmu jaringan dan imunohistokimia. Jakarta. CV Sagung Seto. 2005. 51 p.
11. Jurenka JS. Anti-inflammatory properties of curcumin, a major constituent of Curcuma longa: a review of preclinical and clinical research. Altern Med Rev. 2009 14(2):141–53.
12. Ravindran J, Prasad S, Aggarwal BB. Curcumin and Cancer Cells: How Many Ways Can Curry Kill Tumor Cells Selectively? AAPS J. 2009;11(3):495–510. https://doi.org/10.1208/s12248-009-9128-x
13. Thomas D. Gilmore. NF-κB and Human Cancer: What Have We Learned over the Past 35 Years. Biology Department, Boston University, Boston, MA 02215, USA. 2021.
14. Genestier L, Paillot R, Fournel S, Ferraro C, Miossec P, Revillard JP. Immunosuppressive properties of methotrexate: apoptosis and clonal deletion of activated peripheral T cells. J Clin Invest. 1998;102(2):322–8. https://doi.org/10.1172/JCI2676
15. Spurlock CF, Tossberg JT, Matlock BK, Olsen NJ, Aune TM. Methotrexate Inhibits NF-κB Activity Via Long Intergenic (Noncoding) RNA-p21 Induction. Arthritis Rheumatol. 2014;66(11):2947–57. https://doi.org/10.1002/art.38805
16. Ying Sun, Yang Liu, Xiaoli Ma, Hao Hu. The Influence of Cell Cycle Regulation on Chemotherapy. Int. J. Mol. Sci. 2021, 22(13), 6923; https://doi.org/10.3390/ijms22136923
17. Neradil J, Pavlasova G, Veselska R. New mechanisms for an old drug; DHFR- and non-DHFR-mediated effects of methotrexate in cancer cells. Klin Onkol. 2012;25 Suppl 2:2S87-92.
18. Mains PE, Sulston IA, Wood WB. Dominant maternal-effect mutations causing embryonic lethality in Caenorhabditis elegans. Genetics. 1990; 125(2):351–69.
19. Nakamura M, Saito H, Ebinuma H, et al. Reduction of telomerase activity in human liver cancer cells by a histone deacetylase inhibitor. J Cell Physiol. 2001;187(3):392-401. https://doi.org/10.1002/jcp.1087
20. Zhu X, Kumart R, Mandalt M, et al. Cell cycle-dependent modulation of telomerase activity in tumor cells. Proc Natl Acad Sci. 1996;93:6091-5.
21. Liu T, Yuan X, Xu D. Cancer-Specific Telomerase Reverse Transcriptase (TERT) Promoter Mutations: Biological and Clinical Implications. Genes (Basel). 2016;7(7):38. https://doi.org/10.3390/genes7070038
22. Molood S, Samira H, Ziba M, Maliheh E, Narges B, Majid S. Nanocurcumin-mediated down-regulation of telomerase via stimulating TGF beta 1 signaling pathway in hepatocellular carcinoma cells. 2018;171–9.
23. Forouzanfar F, Majeed M, Jamialahmadi T, Sahebkar A. Telomerase: a Target for Therapeutic Effects of Curcumin in Cancer. Adv Exp Med Biol. 2021;1286:135-43. doi: 10.1007/978-3-030-55035-6_10
24. Spurlock CF, Gass HM, Bryant CJ, et al,. Methotrexate-mediated inhibition of nuclear factor κB activation by distinct pathways in T cells and fibroblast-like synoviocytes. Rheumatol. 2015 ;54(1):178–87. https://doi.org/10.1093/rheumatology/keu279
25. Majumdar S, Aggarwal BB. Methotrexate Suppresses NF-κB Activation Through Inhibition of IκBαPhosphorylation and Degradation. J Immunol. 2001;167(5):2911–20. https://doi.org/10.4049/jimmunol.167.5.2911
26. Karunaweera N, Raju R, Gyengesi E, MÃ¼nch G. Plant polyphenols as inhibitors of NF-ÎoB induced cytokine productionâ€"a potential anti-inflammatory treatment for Alzheimer's disease? Front Mol Neurosci. 2015 ;8. https://doi.org/10.3389/fnmol.2015.00024
27. Singh S, Aggarwal BB. Activation of Transcription Factor NF-κB Is Suppressed by Curcumin (Diferuloylmethane). J Biol Chem. 1995 ;270(42):24995–5000. https://doi.org/10.1074/jbc.270.42.24995
28. Panda AK, Chakraborty D, Sarkar I, Khan T, Sa G. New insights into therapeutic activity and anticancer properties of curcumin. J Exp Pharmacol. 2017;9:31–45. https://doi.org/10.2147/JEP.S70568
29. Leif RC, Stein JH, Zucker RM. A short history of the initial application of anti-5-BrdU to the detection and measurement of S phase. Cytometry. 2004;58A(1):45–52. https://doi.org/10.1002/cyto.a.20012
30. Lim W, Jeong M, Bazer FW, Song G. Curcumin Suppresses Proliferation and Migration and Induces Apoptosis on Human Placental Choriocarcinoma Cells via ERK1/2 and SAPK/JNK MAPK Signaling Pathways. Biol Reprod. 2016;95(4):83. https://doi.org/10.1095/biolreprod.116.141630