Original Article

Potential of AKNA as a Predictive Biomarker for Ovarian Cancer and Its Relationship to Tumor Grading

P Rustamadji, E Wiyarta[1], M Miftahuzzakiyah, D Sukmawati[2], DA Suryandari[3], R Kodariah

Departments of Anatomic Pathology, [1]Medical Science, [2]Histology, and [3]Medical Biology, Faculty of Medicine Universitas Indonesia-Dr. Cipto Mangunkusumo National Hospital, Jakarta, Indonesia

Received: 12-Jan-2024;
Revision: 04-Aug-2024;
Accepted: 14-Aug-2024;
Published: 30-Sep-2024

ABSTRACT

Background: Ovarian cancer exhibits a significant prevalence and incidence on a global scale. Low-grade or high-grade epithelial-type ovarian cancer can be classified by using the dualistic model. Inflammation has been associated with AKNA protein by cancer researchers. The potential of AKNA as a cancer biomarker is supported by its significance and association with ovarian carcinoma. Uninvestigated is this enormous potential. **Aim:** This study examines the correlation between AKNA expression in low-grade and high-grade ovarian tumors and its utility as a predictive biomarker for ovarian cancer. **Methods:** This study examined a total of thirty-one samples, which were classified into three groups: cyst, low-grade, and high-grade ovarian carcinoma. The departmental archive was accessed for the following information: age, tumor size, nuclear grade, mitosis, ovary volume, implant tumor status, lymph vascular invasion status, lymph node metastasis, and tumor-infiltrating lymphocyte. The expression of AKNA was determined using IHC staining. The information was collected and analyzed via analysis of variance. **Results:** The AKNA H-score shows the mean difference between all three groups ($P < 0.001$). Cysts had the highest AKNA expression, followed by low-grade and high-grade ovarian carcinoma. **Conclusion:** Higher-grade ovarian cancer expressed less AKNA compared to cysts or low-grade forms of the disease. This considerable difference suggests that AKNA might predict ovarian cancer tumor grade.

KEYWORDS: *AKNA, dualistic model, ovarian carcinoma, ovarian cyst, tumor grade*

INTRODUCTION

Ovarian cancer is a substantial global health issue characterized by elevated incidence and prevalence rates. As the most prevalent gynecologic cancer in 2018, ovarian cancer accounted for an estimated 295,414 newly diagnosed cases and 184,799 fatalities.[1] The American Cancer Society estimates that about 1 in 78 women in the USA will develop ovarian cancer at some point during their lifetime.[2] Morbidity, or the rate of illness, is also high for ovarian cancer, as it is often not diagnosed until later stages when treatment options are limited, and the chance of survival is lower.[3] The 5-year survival rate for women with ovarian cancer is only 48.6%.[4] This finding highlights the importance of early detection and timely intervention for ovarian cancer patients.

Access this article online

Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/njcp.njcp_46_24

Epithelial-type ovarian cancer is categorized into two distinct categories, low-grade and high-grade, according to the dualistic model.[5] Low-grade ovarian cancer is characterized by its early-stage nature, infrequent occurrence of ascites, overall favorable prognosis, and a significant likelihood of endometriosis development.[5] Although the high-grade type is typically identified by the presence of a P53 mutation, the majority of these

Address for correspondence: Dr. P Rustamadji, Department of Anatomical Pathology, Cipto Mangunkusumo Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta 10430, Indonesia.
E-mail: primariadewi.rustamadji@ui.ac.id, primariadewi@yahoo.com

How to cite this article: Rustamadji P, Wiyarta E, Miftahuzzakiyah M, Sukmawati D, Suryandari DA, Kodariah R. Potential of AKNA as a predictive biomarker for ovarian cancer and its relationship to tumor grading. Niger J Clin Pract 2024;27:1089-94.

tumors are advanced ovarian malignancies with high proliferation, BRCA mutations, a poor prognosis, and ascites.[5] The dualistic model facilitates the differentiation and identification of cellular and molecular processes that transpire during the early and advanced stages of ovarian cancer pathogenesis.[6] Ovulation is a contributing factor to the development of ovarian cancer. During the tissue repair phase that follows ovulation, an excessive inflammatory microenvironment, genetic alterations, and uncontrolled epithelial-mesenchymal transition (EMT)—which initiates the metamorphosis of epithelial cells—are present.[6,7] During the initiation phase of tumorigenesis, the immune system and other biological responses are implicated in the elimination of cancer antigens.[8] AKNA is one of the proteins involved in the regulation of EMT, anti-tumor immune response, and inflammation.[9]

AKNA protein has been studied in cancer and has been shown to play a role in the inflammatory response.[10] The absence of AKNA can stimulate inflammatory dysregulation, increased MMP-9 proteolysis, and inflammatory cytokines.[11,12] This mechanism shows that the AKNA protein regulates the balance of inflammation by protecting various factors that can damage the effects of inflammation, preventing the occurrence of immune escape, chronic inflammation, and excessive proliferation in cancer.[13] The AKNA protein plays an essential role in regulating the balance of the inflammatory response, the AKNA transcription factor can bind directly to the AT-rich promoter region, so the AKNA gene that encodes the AKNA transcription factor can also bind to the AT-rich promoter region of the IL-10 gene.[13,14] Thus, the AKNA gene has an essential role in enhancing the immune response, inflammation, carcinogenesis, and regulation of EMT. AKNA dysregulation can increase carcinogenesis and cancer progression, which is suspected in ovarian cancer.

AKNA has the potential to function as a biomarker for carcinoma, given its significance and correlation with the carcinogenic process of ovarian carcinoma. However, this immense potential remains unexplored. To address this knowledge gap, we conducted this study which aims to examine the correlation between AKNA expression in low-grade and high-grade ovarian tumors and its utility as a predictive biomarker for ovarian cancer. A reduced level of AKNA expression was hypothesized in high-grade ovarian carcinoma compared to low-grade carcinoma.

Materials and Methods

Study design and data collection

This retrospective cohort study was conducted at the Department of Anatomical Pathology, Faculty of Medicine, University of Indonesia. In May 2022, the Faculty of Medicine's Institutional Review Board (IRB) at the University of Indonesia authorized experimental procedures with protocol number ND-344/UN2.F1/ETIK/PPM.00.02/2022. The research adheres to the World Medical Association's Code of Ethics (Declaration of Helsinki).[15] Samples were taken from Biobank departmental archives from September 2016 to December 2022. The population of this study was all preparations of hematoxylin-eosin (HE) and paraffin block in female patients who had been histopathologically diagnosed with high-grade and low-grade ovarian carcinoma. As a control sample, patients with cysts were included in the analysis. We excluded specimens from patients with pathologies other than ovarian carcinoma, those with systemic comorbidities (diabetes and hypertension), and those with questionable paraffin blocks (e.g. broken or weakened paraffin blocks).

Sample selection was carried out by consecutive sampling, namely collecting as many samples as possible from archives from September 2016 to December 2022. The sample size is calculated based on a 95% confidence level or 5% alpha and 80% power or 20% beta. Patient age, tumor site, nuclear grade, mitosis, ovary volume, implant tumor status, lymphovascular invasion status (LVI), tumor-infiltrating lymphocyte (TIL), and lymph node metastasis (LNM) were gathered from the departmental archive. The volume of ovarian tissue submitted for histological examination was accomplished using a water displacement method, where the tissue specimen was submerged in a graduated cylinder containing a known volume of water. In addition, data on the expression of AKNA were quantified using immunohistochemistry.

Slide preparation

According to the protocol of Kusmardi *et al*.[16,17] and Rustamadji *et al*.,[18,19] AKNA protein analysis using the immunohistochemistry method used tonsil tissue as a positive control. The primary antibody dilution used was 1:100. Then, an analysis was carried out on samples of ovarian cancer formalin-fixed paraffin-embedded (FFPE) tissue. Ovarian cancer tissue was cut 2.5 μm and then dried at 37°C. The tissue pieces were then heated over a slide warmer at 60°C for 30 minutes. Furthermore, the sample deparaffinization was carried out by inserting the sample slide into xylol (Brataco Inc., Jakarta, Indonesia) I, II, and III for 3 minutes. Then rehydrate the sample slide for 3 minutes into graded alcohol (Brataco Inc., Jakarta, Indonesia), namely absolute alcohol, 96% alcohol, and 70% alcohol by order. Then it was washed with distilled water for 3 minutes. Then, 3% peroxide endogenous (Merck, Jakarta, Indonesia) blocking was

performed for 10 minutes. Wash under running water for 5 minutes. After that, antigen retrieval was carried out by autoclaving at 96°C for 20 minutes. Refrigerate for 25 minutes. Sample slides were washed using phosphate buffer saline (Merck, Jakarta, Indonesia) pH 7.4 for 5 minutes and then continued with background sniper blocking for 15 minutes. The slides were incubated with anti-AKNA primary antibody (Fine-Test, China) onto each slide at room temperature overnight. After incubation, the slides were washed with phosphate buffer saline pH 7.4 for 2 minutes, then incubated with anti-anti-AKNA secondary antibody (Merck, Jakarta, Indonesia) at room temperature for 20 minutes.

The incubated slides were then washed with phosphate buffer saline pH 7.4 for 2 minutes and incubated with trekAvidin-HRP (Merck, Jakarta, Indonesia) at room temperature for 15 minutes. Then washed with phosphate buffer saline pH 7.4 and applied DAB substrate kit (Merck, Jakarta, Indonesia) at room temperature for 1–2 minutes and then washed with running water for 2 minutes. Then counterstained with hematoxylin (Brataco Inc., Jakarta, Indonesia) for 10 seconds and washed with running water for 3 minutes. Then, 5% saturated lithium carbonate (Brataco Inc., Jakarta, Indonesia) was given in distilled water for 5 seconds and washed with running water for 2 minutes. The slides were then dehydrated with graded alcohol (Brataco Inc., Jakarta, Indonesia), namely 80% alcohol, 96% alcohol, and absolute alcohol, by order for 3 minutes each. Then clearing with xylol (Brataco Inc., Jakarta, Indonesia), I, II, and III were carried out for 3 minutes each. Mounted and observed using a microscope.[20]

AKNA quantification

Immunohistochemical results on the slide samples were observed using a microscope. Immunohistochemical methods can detect the presence and expression levels of proteins. Five visual fields were observed, with a maximum of 100 cells per visual field at 40 × magnification. Images were processed using the fijiwin 32 application from the ImageJ application, which can calculate the percentage and intensity. First, the image was entered into the application. Then the plugin menu was selected, analyze cell counter was, and then the nuclei were selected to be analyzed.[21] Then the score was calculated using a modified Histo-score (H-score), namely a score of 3 for strong positive nuclei, 2 for moderate positive nuclei, and 0 for negative nuclei.[22,23]

Statistical analysis

Microsoft Excel entered data collection into the primary table before analysis (Microsoft Corp, Redmond, WA, USA). Version 20 of Statistical Package for the Social Sciences (SPSS) was used for analysis and visualization (IBM Corp, Armonk, NY, USA). For univariate analysis, all categorical variables are represented as numbers (percentages) and analyzed using Fisher's exact test, while all numerical variables are expressed as means (standard deviation) and analyzed with one-way analysis of variance (ANOVA).

Results

In total, there were 31 samples examined in this study, consisting of cyst, low-grade, and high-grade ovarian carcinoma groups. According to the clinicopathological features of the participants in Table 1, age had no bearing on the grading level of ovarian cancer cases. A notable distinction in tumor location was observed between high grade (bilateral) and low grade (unilateral). In comparison with the control group, nuclear grade and mitotic factors varied substantially between the high-grade and low-grade groups. This finding indicates that moderate to severe nuclear grades are more prevalent in cases classified as low-grade and high-grade, as opposed to mild nuclear grades. A decrease in mitotic activity is observed in lower grades, followed by an increase in activity in higher grades. In high-grade cases, the average ovarian volume decreased considerably in comparison with the control group and low-grade cases. We observed a significant difference in the diminution of the AKNA H-score value between high-grade, control, and low-grade tumors, indicating that AKNA expression is involved in the grading of ovarian carcinoma ($P < 0.001$).

Figure 1 shows immunohistochemistry results in samples with positive AKNA staining compared to negative AKNA in each cyst sample, low-grade ovarian carcinoma, and high-grade ovarian carcinoma. AKNA expression is shown in brown in the sample. The darker the brown color, the stronger the AKNA expression in the sample. Because of the color intensity, the AKNA expression can be quantified using the H-score. These

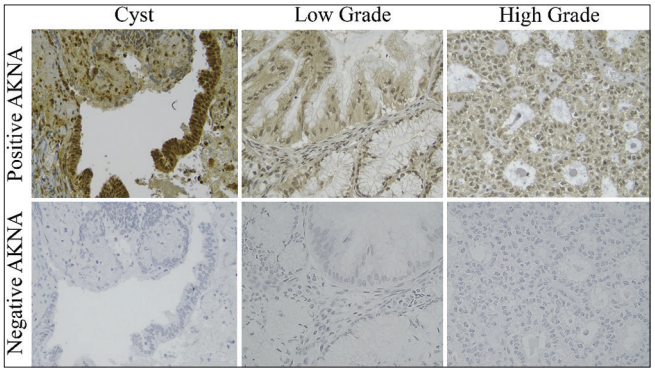

Figure 1: AKNA and TIL immunohistochemistry on control, low-grade, and high-grade ovarian cancer. The scale bar represents 50 μm for all images

Table 1: Clinicopathological characteristics of the samples

Characteristics	Control (*n*=6)	Low grade (*n*=12)	High grade (*n*=13)	*P*
Age (years)	47.00 (4.19)	45.08 (2.91)	44.31 (1.78)	0.824
Tumor site				
Unilateral	5.00 (31.25%)	10.00 (62.50%)	1.00 (6.25%)	<0.001*
Bilateral	1.00 (6.70%)	2.00 (13.30%)	12.00 (80.00%)	
Nuclear grade				
Moderate-severe	1.00 (3.80%)	12 (46.20%)	13.00 (50.00%)	<0.001*
Mild	5.00 (100.00%)	0.00 (0.00%)	0.00 (0.00%)	
Mitosis				
High activity	0.00 (0.00%)	0.00 (0.00%)	9.00 (100.00%)	<0.001*
Low activity	6.00 (27.30%)	12.00 (54.50%)	4.00 (18.20%)	
Ovary volume (cm^3)				
Right	528.48 (474.77)	2665.64 (1614.32)	287.47 (156.93)	0.230
Left	1273.77 (700.57)	1698.55 (490.89)	451.24 (264.85)	0.110
Mean	901.13 (336.19)	2182.09 (711.78)	369.36 (208.04)	0.034*
Implant tumor				
Yes	4.00 (25.00%)	4.00 (25.00%)	8.00 (50.00%)	0.326
No	2.00 (13.33%)	8.00 (53.33%)	5.00 (33.33%)	
LVI				
Yes	2.00 (18.20%)	2.00 (18.20%)	7.00 (63.60%)	0.183
No	4.00 (20.00%)	10.00 (50.00%)	6.00 (30.00%)	
TIL				
High	0.00 (0.00%)	2.00 (25.00%)	6.00 (75.00%)	0.075
Low	6.00 (26.10%)	10.00 (43.50%)	7.00 (30.40%)	
LNM				
Yes	1.00 (14.30%)	1.00 (14.30%)	5.00 (71.40%)	0.217
No	5.00 (20.80%)	11.00 (45.80%)	8.00 (33.33%)	
AKNA H-score	268.12 (8.70)	117.47 (20.46)	23.17 (4.37)	<0.001*

**P*-value less than 0.05 is considered statistically significant. Data are means (SD) or numbers (%). LNM: lymph node metastasis; LVI: lymph vascular invasion; SD: standard deviation; TIL: tumor-infiltrating lymphocyte

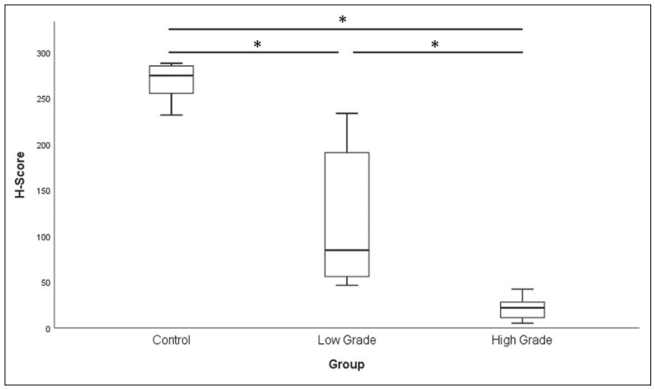

Figure 2: Comparison of AKNA H-score in each group. **P* value less than 0.05 is considered statistically significant

differences can then be seen in Figure 2, which shows the boxplot of the differences in AKNA H-scores in each group. All groups have a significant mean difference from one another. Cysts had the highest AKNA expression, followed by low-grade and high-grade ovarian carcinoma.

Discussion

This study shows that AKNA has significantly different expression between ovarian cyst, low-grade, and high-grade ovarian carcinoma groups. This difference can be seen visually on the microscopic slide [Figure 1] and quantitatively through the H-score [Figure 2]. The highest AKNA expression was in the cyst group, while the lowest was in the high-grade ovarian carcinoma group. This finding happens because it is related to the mechanism of AKNA and its role in the inflammatory process.

The AKNA gene is on chromosome 9q32 and is composed of 31 exons.[10] The AT-Hook Transcription Factor encoded by the AKNA gene is a nuclear protein component.[15] AKNA is highly expressed in the ovaries and the germinal center of secondary lymphoid organs during the development of B cells, immunological cells including B cells, T cells, natural killer (NK) cells, dendritic cells, and epithelial cells.[11] The AKNA transcription factor protein has a 9-amino acid domain termed AT-Hook DNA Binding.[15] The AKNA gene is found in the FRA9E region, which is related to inflammation and neoplastic disorders and is prone to mutations.[24,25] It is known that FRA9E is a Fragile Common Site (FCS) that is susceptible to mutations and

instability.[25,26] This finding may occur at the beginning of cancer initiation or precancer, promoting the creation of precancerous lesions, such as those seen in the colon, bladder, and ovarian cancer.[27] In ovarian cancer, the AKNA gene is known to be a region related to loss of heterozygosity (LOH) and occurs in >50% of cases.[25]

AKNA transcription factors also play a role in repressing the expression of inflammatory genes encoding cytokines, proteases, and chemotactic factors. This hypothesis is supported by research that the expression of MMP-9, Interferon 1β, and neutrophil granule protein increases and affects alveolar damage in AKNA knockout mice.[11] The absence of AKNA can stimulate inflammatory dysregulation, increased MMP-9 proteolysis, and inflammatory cytokines.[17,28] This finding shows that the AKNA protein regulates the balance of inflammation by protecting various factors that can damage the effects of inflammation, preventing immune escape, chronic inflammation, and excessive proliferation in cancer.[11,12] AKNA protein plays an essential role in regulating the balance of the inflammatory response. AKNA transcription factors can bind directly to the AT-rich promoter region. The AKNA gene, which encodes the AKNA transcription factor, can also bind to the promoter AT-rich region of the IL-10 gene. It is known that the IL-10 gene, which encodes the IL-10 protein, regulates inflammation, thereby preventing chronic inflammation by regulating the effector function of macrophage cells, T cells, and NK cells. Therefore, the AKNA gene can induce IL-10 expression and increase B-cell initiation by Epstein–Barr virus in B-cell lymphoma cancer.[29] While in cervical cancer, there is a decrease in AKNA expression so that AKNA dysregulation can increase cancer progression, decreased AKNA expression is also associated with decreased p53, thereby increasing cervical cancer progression.[13]

It was believed that AKNA eliminated cancer antigens through an inflammatory response; however, dysregulation of AKNA led to an increase in cancer-related chronic inflammation. Subsequent research discovered that the AKNA protein produced by the AKNA gene is also expressed in epithelial cells; the lack of AKNA in epithelial cells results in a weakened state of cell homeostasis.[14] Therefore, epithelial cells contribute to uncontrolled EMT. The AKNA protein becomes a tumor suppressor in gastric cancer by limiting metastasis through EMT suppression.[14] In gastric cancer, dysregulation of AKNA expression produces an imbalance between anti-tumor and pro-tumor responses, activating chemokine and JAK-STAT signaling pathways.[30] According to the Gene Set Enrichment Analysis (GSEA), AKNA controls cell-cell adhesion and EMT-related pathways.[31] AKNA is a susceptibility gene for cancer, as shown by studies including genetic variation in AKNA.[10] Previous research has shown that the AKNA SNP rs10817595 (-1372C>A) is linked to squamous intraepithelial lesion (SIL) and cervical cancer.[32] The AKNA promoter SNP may influence the expression of the AKNA gene at the transcriptional level, influencing the translation process and transcription factor proteins.[33] In addition, it may have ramifications for its action, which modifies the expression of genes associated with inflammation, carcinogenesis, and the advancement of cancer.[33] According to previous research, SNP -1372C>A is also related to osteoarthritis in the Chinese population.[34]

All of the processes described above explain the involvement of AKNA in the development of ovarian cancer. These numerous processes explain why AKNA expression is lower in patients with high-grade ovarian cancer compared to low-grade or even cysts. The presence of variances in the expression of this essential AKNA can be employed as a predictive biomarker to distinguish tumor grade in ovarian cancer.

The limitation of this study is the restricted sample size employed. Additional research utilizing larger sample sizes is required to obtain greater confidence in the data and a more complete understanding of AKNA's capability as a predictor marker for ovarian tumor differentiation grading.

Conclusion

AKNA expression was less in higher-grade ovarian carcinoma compared to ovarian cysts or low-grade carcinoma. This significant difference indicates that AKNA has the potential to be a predictive biomarker to differentiate tumor grading in ovarian carcinoma.

Ethics approval

Samples were acquired from the tissue archive of the Biobank, which is housed within the Department of Anatomical Pathology of the Medical Faculty at the University of Indonesia. The Ethics Committee of the Faculty of Medicine, the University of Indonesia, approved the report protocols, with protocol number ND-344/UN2.F1/ETIK/PPM.00.02/2022, in May 2022.

Consent for publication

Informed consent for publication was obtained from all subjects involved in the study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, *et al*. Ovarian cancer statistics, 2018. CA Cancer J Clin 2018;68:284-96.
3. Budiana ING, Angelina M, Pemayun TGA. Ovarian cancer: Pathogenesis and current recommendations for prophylactic surgery. J Turk Ger Gynecol Assoc 2019;20:47-54.
4. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020;70:7-30.
5. Kurman RJ, Shih I-M. The dualistic model of ovarian carcinogenesis: Revisited, revised, and expanded. Am J Pathol 2016;186:733-47.
6. Matulonis UA, Sood AK, Fallowfield L, Howitt BE, Sehouli J, Karlan BY. Ovarian cancer. Nat Rev Dis Primers 2016;2:16061.
7. Saad AF, Hu W, Sood AK. Microenvironment and pathogenesis of epithelial ovarian cancer. Horm Cancer 2010;1:277-90.
8. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, *et al*. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 2018;9:7204-18.
9. Moliterno AR, Resar LMS. AKNA: Another AT-hook transcription factor "hooking-up" with inflammation. Cell Res 2011;21:1528-30.
10. Ramírez-González A, Manzo-Merino J, Contreras-Ochoa CO, Bahena-Román M, Aguilar-Villaseñor JM, Lagunas-Martínez A, *et al*. Functional role of AKNA: A scoping review. Biomolecules 2021;11:1709.
11. Moliterno AR, Resar LM. AKNA: Another AT-hook transcription factor "hooking-up" with inflammation. Cell Res 2011;21:1528-30.
12. Ma W, Ortiz-Quintero B, Rangel R, McKeller MR, Herrera-Rodriguez S, Castillo EF, *et al*. Coordinate activation of inflammatory gene networks, alveolar destruction and neonatal death in AKNA deficient mice. Cell Res 2011;21:1564-77.
13. Manzo-Merino J, Lagunas-Martínez A, Contreras-Ochoa CO, Lizano M, Castro-Muñoz LJ, Calderón-Corona C, *et al*. The human papillomavirus (HPV) E6 oncoprotein regulates CD40 expression via the AT-hook transcription factor AKNA. Cancers (Basel) 2018;10:521.
14. Camargo Ortega G, Falk S, Johansson PA, Peyre E, Broix L, Sahu SK, *et al*. The centrosome protein AKNA regulates neurogenesis via microtubule organization. Nature 2019;567:113-7.
15. World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. JAMA 2013;310:2191-4.
16. Kusmardi K, Wiyarta E, Estuningtyas A, Sahar N, Midoen YH, Tedjo A. Potential of Phaleria macrocarpa leaves ethanol extract to upregulate the expression of caspase-3 in mouse distal colon after dextran sodium sulphate induction. Pharmacogn J 2021;13:23-9.
17. Kusmardi K, Wiyarta E, Estuningtyas A, Sahar N, Midoen YH, Tedjo A, *et al*. Potential inhibition by Phaleria macrocarpa leaves ethanol extract on Ki-67 expression in distal colon mouse. Pharmacogn J 2021;13:443-9.
18. Rustamadji P, Wiyarta E, Bethania KA, Kusmardi K. Potential of AKT2 expression as a predictor of lymph-node metastasis in invasive breast carcinoma of no special type. J Pathol Transl Med 2021;55:271-8.
19. Rustamadji P, Wiyarta E, Bethania KA. CD44 variant exon 6 isoform expression as a potential predictor of lymph node metastasis in invasive breast carcinoma of no special type. Int J Breast Cancer 2021;2021:1586367.
20. Wiyarta E, Kusmardi K, Tedjo A, Sunaryo H. *In vitro* and *in vivo* study of Pandanus conoideus oil extract in the maturation of mouse peritoneal macrophages. Open Access Maced J Med Sci 2022;10:419-25.
21. Wiyarta E, Kusmardi K, Midoen YH. Effect of omega-3-rich fish oil on TNF-α expression in mice's colonic tissue induced with azoxymethane (AOM) and dextran sodium sulphate (DSS). Res J Pharm Technol 2022;15:3179-84.
22. Kusmardi K, Wiyarta E, Rusdi NK, Maulana AM, Estuningtyas A, Sunaryo H. The potential of lunasin extract for the prevention of breast cancer progression by upregulating E-Cadherin and inhibiting ICAM-1. F1000Res 2021;10:902.
23. Rustamadji P, Wiyarta E, Bethania KA. Exploring the integrated role of AKT2, CD44v6, and MT1-MMP as predictors of axillary lymph node metastasis in invasive breast carcinoma of no special type. Iran J Pathol 2022;17:480-90.
24. Ball NJ, McGeehan JE, Streeter SD, Thresh SJ, Kneale GG. The structural basis of differential DNA sequence recognition by restriction-modification controller proteins. Nucleic Acids Res 2012;40:10532-42.
25. Callahan G, Denison SR, Phillips LA, Shridhar V, Smith DI. Characterization of the common fragile site FRA9E and its potential role in ovarian cancer. Oncogene 2003;22:590-601.
26. Ma K, Qiu L, Mrasek K, Zhang J, Liehr T, Quintana LG, *et al*. Common fragile sites: Genomic hotspots of DNA damage and carcinogenesis. Int J Mol Sci 2012;13:11974-99.
27. Baker KT, Salk JJ, Brentnall TA, Risques RA. Precancer in ulcerative colitis: The role of the field effect and its clinical implications. Carcinogenesis 2018;39:11-20.
28. Manicone AM, McGuire JK. Matrix metalloproteinases as modulators of inflammation. Semin Cell Dev Biol 2008;19:34-41.
29. Niller HH, Szenthe K, Minarovits J. Epstein-Barr virus-host cell interactions: An epigenetic dialog? Front Genet 2014;5:367.
30. Chen W, Wu G, Zhu Y, Zhang W, Zhang H, Zhou Y, *et al*. HOXA10 deteriorates gastric cancer through activating JAK1/STAT3 signaling pathway. Cancer Manag Res 2019;11:6625-35.
31. Wang G, Sun D, Li W, Xin Y. AKNA is a potential prognostic biomarker in gastric cancer and function as a tumor suppressor by modulating EMT-related pathways. Biomed Res Int 2020;2020:6726759.
32. Martínez-Nava GA, Torres-Poveda K, Lagunas-Martínez A, Bahena-Román M, Zurita-Díaz MA, Ortíz-Flores E, *et al*. Cervical cancer-associated promoter polymorphism affects akna expression levels. Genes Immun 2015;16:43-53.
33. Siddiqa A, Sims-Mourtada JC, Guzman-Rojas L, Rangel R, Guret C, Madrid-Marina V, *et al*. Regulation of CD40 and CD40 ligand by the AT-hook transcription factor AKNA. Nature 2001;410:383-7.
34. Zhao T, Ma C, Xie B, Zhao B, Wang W, Liu J. Evaluation of common variants in the AKNA gene and susceptibility to knee osteoarthritis among the Han Chinese. Genet Test Mol Biomarkers 2020;24:425-30.