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The Frequency of the v-AKT Murine Thymoma Viral Oncogene Homologue 1 Gene Amplification among Sudanese Women with Ovarian Cancer: A Cross-Sectional Study

BMC Research Notes

Dear Dr Akrom,

Thank you very much for your review of manuscript RESN-D-20-01932R1, 'The Frequency of the v-AKT Murine Thymoma Viral Oncogene Homologue 1 Gene Amplification among Sudanese Women with Ovarian Cancer: A Cross-Sectional Study'.

We greatly appreciate your assistance.

Best wishes,

Iman Tavassoly

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The Frequency of the v-AKT Murine Thymoma Viral Oncogene Homologue 1 Gene Amplification among Sudanese Women with Ovarian Cancer: A Cross-Sectional Study

--Manuscript Draft--

Manuscript Number:	RESN-D-20-01932R1
Full Title:	The Frequency of the v-AKT Murine Thymoma Viral Oncogene Homologue 1 Gene Amplification among Sudanese Women with Ovarian Cancer: A Cross-Sectional Study
Article Type:	Research note
Abstract:	Abstract Objectives: Protein kinase B (AKT/PKB) family is frequently altered in ovarian cancer (OC). In Sudan, there is a scarcity of published information about the AKT1 gene in Sudanese women with OC. The study was conducted to detect the AKT1 gene amplification and its association with tumour types, grades, and ages in Sudanese women suffering from OC. Results: This a retrospective, cross sectional study comprised 79 cases of OC diagnosed women. The mean age (±SD) of involved women was 49.29 (± 13.612). Out of seventy-nine cases, amplification was showed in (22.8%) of OC women. It was noted in (50%) of undifferentiated, (33.3%) clear cell, (27.3%) mucinous, (17.6%) endometrioid, and (16.7%) serous carcinomas. The high frequency was seen in women with low (26.3%) than in higher (19.5%) grade carcinoma, and in older (25.8%) than younger (18.2%) women. The study recognized AKT1 gene amplification in about one-fifth of cases. It is seen more in the undifferentiated and clear cell types. Also, we found that amplification had a role in the progress of the early grade of ovarian cancer. These outcomes reveal the role of AKT1 gene in OC progression and might offer a strategy for treatment and prognostic assessment of OC patients.
Response to Reviewers:	greeting please kindly find my revised paper

The Frequency of the v-AKT Murine Thymoma Viral Oncogene Homologue 1 Gene Amplification among Sudanese Women with Ovarian Cancer: A Cross-Sectional Study

Rawia Eljaili Elmassry[1], Aisha Osman Mohammed[1], Nassr Eldin M. A. Shrif[2], Lubna Elnajeb[1], Nazik Elmalaika Husain[3].

Abstract

Objectives: Protein kinase B (AKT/PKB) family is frequently altered in ovarian cancer (OC). In Sudan, there is a scarcity of published information about the AKT1 gene in Sudanese women with OC. The study was conducted to detect the AKT1 gene amplification and its association with tumour types, grades, and ages in Sudanese women suffering from OC.

Results: This a retrospective, cross sectional study comprised 79 cases of OC diagnosed women. The mean age (±SD) of involved women was 49.29 (± 13.612). Out of seventy-nine cases, amplification was showed in (22.8%) of OC women. It was noted in (50%) of undifferentiated, (33.3%) clear cell, (27.3%) mucinous, (17.6%) endometrioid, and (16.7%) serous carcinomas. The high frequency was seen in women with low (26.3%) than in higher (19.5%) grade carcinoma, and in older (25.8%) than younger (18.2%) women. The study recognized AKT1 gene amplification in about one-fifth of cases. It is seen more in the undifferentiated and clear cell types. Also, we found that amplification had a role in the progress of the early grade of ovarian cancer. These outcomes reveal the role of AKT1 gene in OC progression and might offer a strategy for treatment and prognostic assessment of OC patients.

Keywords: AKT1 gene, amplification, grade, ovarian cancer, age, q-PCR, targeted therapy, prognosis.

Introduction

Ovarian cancer (OC) is considered a fatal disease among gynaecological cancers. Annually, it marked around 0.2 million ladies worldwide and about 0.125 million deaths (1). The deadly type of OC is epithelial ovarian cancer (EOC). The majority (>75%) of patients are typically diagnosed in advanced stage (2) as of unfortunate early discovery, recurrence, and further metastasis. For these reasons, the prognosis keeps on the poor, the mortality rate is the top, and the five-year survival rate of advanced-stage patients is nearby 30% (3). Chemotherapy, the first-line treatment for OC, composed mainly of platinum and taxane, does not affect overall survival (4). Despite most patients firstly respond to cytoreductive surgery and platinum-based chemotherapies; still, several ultimately progress chemo-resistant tumours, relapse, and die (5).

The serine/threonine kinase (AKT), also identified as protein kinase B (PKB), is an oncogenic family of proteins with a molecular weight ~60 kDa (6). It controls cell growth, survival, proliferation, glycogen metabolism (7), protein synthesis, genome constancy, and apoptosis in response to diverse growth factors and extracellular stimuli (8). There are three types of AKT, termed PKBα (AKT1), PKBβ (AKT2), and PKBγ (AKT3), and all AKT types are linked with cancer progress (9). Among them, amplification and over-expression of AKT2 are frequently detected in OC (6). Akt1 is activated indirectly by insulin and growth factors and is downstream of the phospholipid kinase PI3-K (10). PDK1 and mTORC2 phosphorylate AKT1 at the threonine 308 and 473 sites, respectively (11), while PIP3 recruits it to the membrane (12).

In cancer, AKT is often activated through a range of mechanisms, comprising amplification of growth factor receptors (e.g., HER2/neu and EGFR) (13), amplification or mutation of phosphatidylinositol 3-

kinase (PI3K), and amplification or mutation of AKT isoforms. All these mechanisms can be blocked, so AKT has been widely discovered as an anticancer target therapy (14).

Main text

Material and methods

This retrospective, cross-sectional study was comprised of seventy-nine (79) adult women diagnosed with OC at Omdurman Maternity Hospital between 2013 and 2018. The included OC cases were adults with histologically confirmed ovarian cancer. The socio-demographic data were retrieved from the patients' records and were collected in the period "2016 and 2018".

RNA extraction, cDNA synthesis, and quantitative real-time PCR (q-PCR)

The strategy for total RNA extraction and cDNA production and storage was finished in a similar manner that was referenced in our past inspection. Also, for q-PCR, the convention and machine were utilized equally like a similar strategy in our previous study (15). The Primers were (AKT1 F, 5'-ACGGGCACATTAAGATCACA-3'); R, 5' TGCCGCAAAAGGTCTTCATG-3') and (LINE1 F, 5'CCGCTCAACTACATGGAAACTG-3'); R, 5'- GCGTCCCAGAGATTCTGGTATG-3'). At that point, the amplification was evaluated by recognizing the relative quantification utilizing the $2^{-\Delta\Delta Ct}$ strategy (16). To decide amplification values greater than 2.0 were estimated to be positive.

Statistical analysis

We use SPSS, version 24 (IBM SPSS) to analysis the results. Descriptive analysis was done for variables (tumour types, grades, and ages of women). Fisher's exact test was completed between AKT1 gene amplification and tumour types, grades, and ages to catch statistical significance. P-value < 0.05 was measured as statistically significant.

Results

This study included seventy-nine (79) ovarian cases, with age range 18-75 years, the median age was 50 years, and the mean (±SD) was 49.29 (± 13.612). Women in the age groups > 50 years were (46.8%), and ≤ 50 years accounted for (39.2%). AKT1 gene amplification was detected in 22.8% of OC women. Figure 1 shows its frequency in each tumour type. The high frequency was seen in women with low (26.3%) rather than in higher (19.5%) grade carcinomas, as seen in Figure.2, and in older (25.8%) rather than younger (18.2%) women. No significant association between AKT1 gene amplification and tumour types, grades, and ages of women was observed (Fisher's Exact test: p = 0.405, 0.593 and 0.851, respectively).

Discussion

The AKT family controls cell survival, growth, proliferation, and apoptosis (7). And its activity is frequently elevated in ovarian cancer (17). In this study, we assessed the AKT1 gene amplification in Sudanese women with OC. We found AKT1 gene amplification in 22.8% OC, although, our study

was disagreed with the previous study described by TCGA analysis (3%) (18), however, is agreed with *Despierre et al*. (26.3%) (19). The AKT has several targets (20) and comprises three different isoforms: AKT1-3, which shares a high degree of structural resemblance and activation (21), encouraging us to compare our results with the AKT2 gene amplification. The frequency of AKT1 amplification in the current study is higher than that of *Cheng et al*.; they reported AKT2 gene amplification in 13% of primary OC (22).

On the other hand, *Altomare et al*. revealed p-AKT overexpression in 68% OC (23), and *Abubaker et al*. identified p-AKT overexpression in 52.1% of OC (24). Regarding tumour types, previous studies determine that AKT activation is common in high-grade, late-stage serous ovarian carcinoma (25-28). On the other hand, our results reveal that high-grade serous carcinoma is harbouring a low frequency of amplification. The lack of consistency in the frequency of AKT1 amplification in OC can be due to differences in sample size, ethnic background, the tumour's biology, and method's sensitivity. Concerning the association of AKT1 gene amplification with tumour grades, the present study agreed with Nakayama et al. (25) in which amplification of AKT2 has been reported in only 18.2% of the high-grade tumours.

Conclusions

The current study showed that AKT1 gene amplification relatively arises in around one-fifth of Sudanese women with OC. It is seen more in undifferentiated and clear cell types. Also, we found that amplification had a role in the progress of the early grade of ovarian cancer, which might offer a strategy for treatment and prognostic assessment of ovarian cancer patients.

Limitations

The small sample size and little data limited our study. Supplementary studies with improved sample size and more added data (e.g., stage at diagnosis, chemotherapy, radiation) can probably verify this study's outcome. The impact of combining other genes, co-existing in the AKT family, should also be inspected.

Ethics approval and consent to participate

The Ethics Group of the Ministry of Health of Khartoum state and Omdurman Maternity Hospital have accepted the study. Conversant permission from the patients was waived. Equally, the patients' personality was anonymized, and exclusive laboratory numbers were used.

Competing interests

Authors declare that they have no competing interests.

Grant information

No grants were elaborated in supporting this work.

Consent for publication

Not applicable.

Availability of data and materials

The datasets of the current study are handy from the corresponding author on rational demand.

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Authors' contributions

Rawia Eljaili Mohammed Elmassry: Data Curation, Formal Analysis, Methodology, Project Management, Writing – Original Draft Preparation

Aisha Osman Mohamed Alkhader: Data Collection, Formal Analysis.

Nasser Aldin Mohammed Ahmed Sharif: Formal Analysis & Review.

Lubna Elnajeb Mohamed Alhassan: Data Collection, Formal Analysis.

Nazik Elmalaika Obaid Seid Ahmed Husain: Project Management, Administration, Visualization, Writing – Review & Editing.

Author details

[1]Department of Histopathology and Cytology, Faculty of Medical Laboratory Sciences, Alzaiem Alazhari University, Khartoum, 11111, Sudan, Email: rose01001@gmail.com, aishaosman2012@gmail.com, lubnaalhassan@yahoo.com.

[2]Department of Clinical Chemistry, Faculty of Medical Laboratory Sciences, Alzaiem Alazhari University, Khartoum, 11111, Sudan, Email: aboamr124@hotmail.com.

[3]Department of Pathology, Faculty of Medicine, Omdurman Islamic University, and Radioisotope Center of Khartoum, Khartoum, 11111, Sudan, Email: nazikhusain@gmail.com.

Correspondence to: Rawia Eljaili Elmassry, Department of Histopathology and Cytology, Faculty of Medical Laboratory Sciences, Alzaiem Alazhari University, Khartoum, 11111, Sudan, Telephone: 00249111999459 – Email: rose01001@gmail.com.

References

1. Gong J, Xing C, Wang LY, Xie SS, Xiong WD. L-Tetrahydropalmatine enhances the sensitivity of human ovarian cancer cells to cisplatin via microRNA-93/PTEN/Akt cascade. J Buon. 2019;24(2):701-8.

2. Liu Z, Zhang S, Hou F, Zhang C, Gao J, Wang K. Inhibition of Ca2+-activated chloride channel ANO1 suppresses ovarian cancer through inactivating PI3K/Akt signaling. International journal of cancer. 2019;144(9):2215-26.

3. Shen F, Zong Z-h, Liu Y, Chen S, Sheng X-j, Zhao Y. CEMIP promotes ovarian cancer development and progression via the PI3K/AKT signaling pathway. Biomedicine & Pharmacotherapy. 2019;114:108787.

4. Majem B, Parrilla A, Jiménez C, Suárez-Cabrera L, Barber M, Marín A, et al. MicroRNA-654-5p suppresses ovarian cancer development impacting on MYC, WNT and AKT pathways. Oncogene. 2019;38(32):6035-50.

5. Wu Y-H, Huang Y-F, Chen C-C, Chou C-Y. Akt inhibitor SC66 promotes cell sensitivity to cisplatin in chemoresistant ovarian cancer cells through inhibition of COL11A1 expression. Cell Death & Disease. 2019;10(4):1-16.

6. Zheng B, Geng L, Zeng L, Liu F, Huang Q. AKT2 contributes to increase ovarian cancer cell migration and invasion through the AKT2-PKM2-STAT3/NF-κB axis. Cellular Signalling. 2018;45:122-31.

7. Song M, Bode AM, Dong Z, Lee M-H. AKT as a Therapeutic Target for Cancer. Cancer research. 2019;79(6):1019-31.

8. Shariati M, Meric-Bernstam F. Targeting AKT for cancer therapy. Expert opinion on investigational drugs. 2019;28(11):977-88.

9. Revathidevi S, Munirajan AK, editors. Akt in cancer: mediator and more. Seminars in cancer biology; 2019: Elsevier.

10. Cole PA, Chu N, Salguero AL, Bae H. AKTivation mechanisms. Current opinion in structural biology. 2019;59:47-53.

11. Ediriweera MK, Tennekoon KH, Samarakoon SR, editors. Role of the PI3K/AKT/mTOR signaling pathway in ovarian cancer: Biological and therapeutic significance. Seminars in cancer biology; 2019: Elsevier.

12. Shi X, Wang J, Lei Y, Cong C, Tan D, Zhou X. Research progress on the PI3K/AKT signaling pathway in gynecological cancer. Molecular medicine reports. 2019;19(6):4529-35.

13. Wang J, Zhao W, Guo H, Fang Y, Stockman SE, Bai S, et al. AKT isoform-specific expression and activation across cancer lineages. BMC cancer. 2018;18(1):742.

14. Saura C, Roda D, Roselló S, Oliveira M, Macarulla T, Pérez-Fidalgo JA, et al. A first-in-human phase I study of the ATP-competitive AKT inhibitor ipatasertib demonstrates robust and safe targeting of AKT in patients with solid tumors. Cancer discovery. 2017;7(1):102-13.

15. Elmassry RE, Shrif NEM, Mohammed AO, Elaagip A, Husain NE. Frequency of the phosphatidylinositol3-kinase, catalytic, α-polypeptide gene amplification in ovarian cancer among Sudanese women: a cross-sectional study. F1000Research. 2019;8(1564):1564.

16. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. methods. 2001;25(4):402-8.

17. Linnerth-Petrik NM, Santry LA, Moorehead R, Jücker M, Wootton SK, Petrik J. Akt isoform specific effects in ovarian cancer progression. Oncotarget. 2016;7(46):74820.

18. Network CGAR. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474(7353):609.

19. Etemadmoghadam D, Bowtell D. AKT1 gene amplification as a biomarker of treatment response in ovarian cancer: Mounting evidence of a therapeutic target. Gynecologic oncology. 2014;135(3):409-10.

20. Kyung HY, Lauring J. Recurrent AKT mutations in human cancers: functional consequences and effects on drug sensitivity. Oncotarget. 2016;7(4):4241.

21. van de Stolpe A. Quantitative Measurement of Functional Activity of the PI3K Signaling Pathway in Cancer. Cancers. 2019;11(3):293.

22. Cheng JQ, Godwin AK, Bellacosa A, Taguchi T, Franke TF, Hamilton TC, et al. AKT2, a putative oncogene encoding a member of a subfamily of protein-serine/threonine kinases, is amplified in human ovarian carcinomas. Proceedings of the National Academy of Sciences. 1992;89(19):9267-71.

23. Altomare DA, Wang HQ, Skele KL, De Rienzo A, Klein-Szanto AJ, Godwin AK, et al. AKT and mTOR phosphorylation is frequently detected in ovarian cancer and can be targeted to disrupt ovarian tumor cell growth. Oncogene. 2004;23(34):5853-7.

24. Abubaker J, Bavi P, Al-Haqawi W, Jehan Z, Munkarah A, Uddin S, et al. PIK3CA alterations in Middle Eastern ovarian cancers. Molecular cancer. 2009;8(1):51.

25. Hanrahan AJ, Schultz N, Westfal ML, Sakr RA, Giri DD, Scarperi S, et al. Genomic complexity and AKT dependence in serous ovarian cancer. Cancer discovery. 2012;2(1):56-67.

26. Yuan ZQ, Sun M, Feldman RI, Wang G, Ma X-l, Jiang C, et al. Frequent activation of AKT2 and induction of apoptosis by inhibition of phosphoinositide-3-OH kinase/Akt pathway in human ovarian cancer. Oncogene. 2000;19(19):2324-30.

27. Cristiano BE, Chan JC, Hannan KM, Lundie NA, Marmy-Conus NJ, Campbell IG, et al. A specific role for AKT3 in the genesis of ovarian cancer through modulation of G2-M phase transition. Cancer Research. 2006;66(24):11718-25.

28. Kurose K, Zhou X-P, Araki T, Cannistra SA, Maher ER, Eng C. Frequent loss of PTEN expression is linked to elevated phosphorylated Akt levels, but not associated with p27 and cyclin D1 expression, in primary epithelial ovarian carcinomas. The American journal of pathology. 2001;158(6):2097-106.

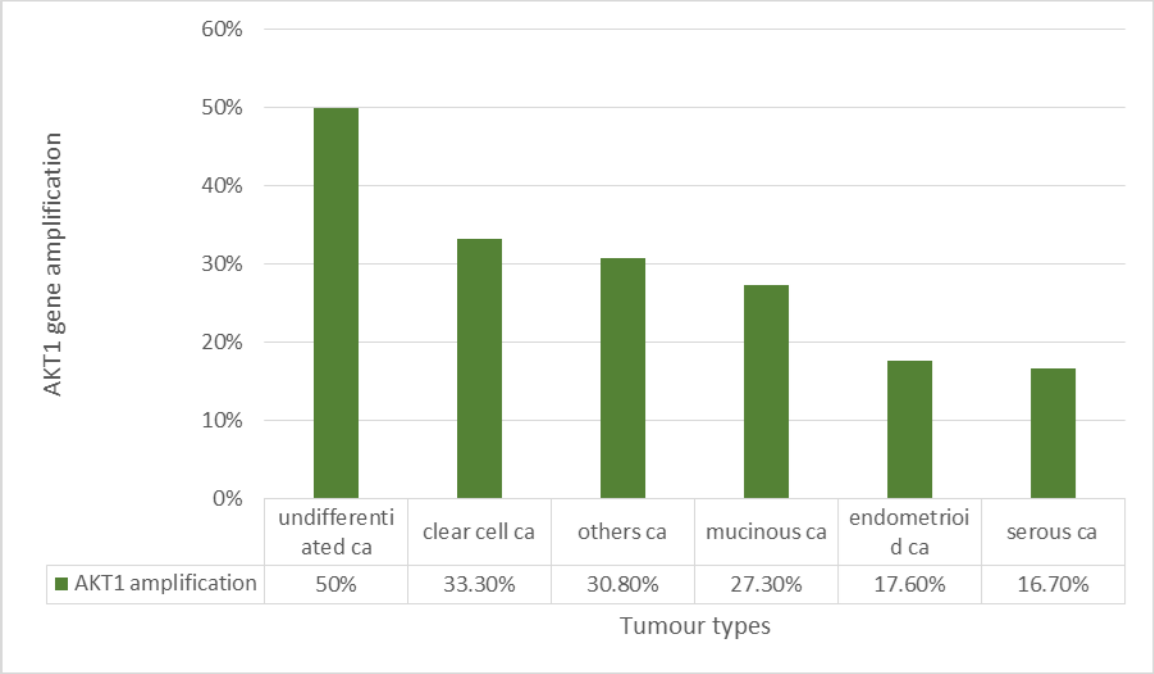

Figure.1: The amplification rate of the v-akt murine thymoma viral oncogene homologue 1 (AKT1) gene amplification rendering to tumour types in ovarian cancer.

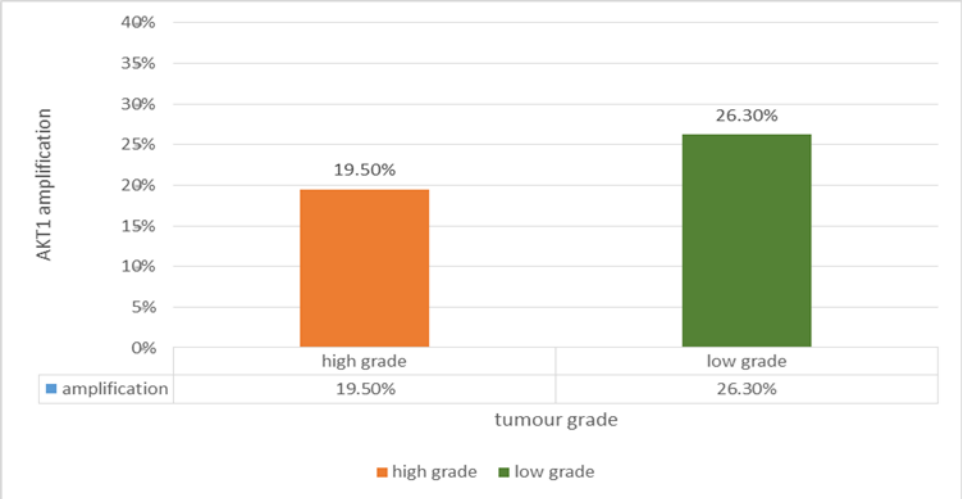

Figure.2: The amplification rate of the v-akt murine thymoma viral oncogene homologue 1 (AKT1) gene rendering to tumour grade in ovarian cancer.