RESEARCH ARTICLE

Frequency of the phosphatidylinositol3-kinase, catalytic, α -polypeptide gene amplification in ovarian cancer among Sudanese women: a cross-sectional study [version 1; peer review: awaiting peer review]

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Abstract

Background: Phosphatidylinositol3-kinase, catalytic, α -polypeptide (PIK3CA) gene is frequently amplified in ovarian carcinoma (OC). To the best of our knowledge, there is a dearth of published reports about the amplification of the PIK3CA gene among Sudanese women with OC. This study aimed to detect the amplification of the PIK3CA gene and its relationship with clinicopathological variables among Sudanese women with OC.

Methods: This cross-sectional study included 90 ovarian cases: 83 cases of women diagnosed with OC at Omdurman Maternity Hospital in the period 2013-2018; 7 cases of women with normal ovarian tissues were used as a control to normalize the results. Formalin-fixed paraffin-embedded tissue sections (FFPE) were used to extract RNA at the Institute of Endemic Diseases, Sudan. PIK3CA gene amplification was assessed using quantitative real-time PCR.

Results: Amplification of PIK3CA was observed in 33.7% (n = 28/83) of women, with a high frequency in women with clear cell (66.7%; n = 4/6), undifferentiated (50.0%; n = 1/2), serous (35.5%; n = 11/31), mucinous (33.3%; n = 4/12), other (30.8%; n = 4/13), and endometrioid (21.1%; n = 4/19) carcinomas. High frequency was seen in women with higher (39.5%; n = 17/43) rather than in lower grade carcinomas (27.5%; n = 11/40), and in older (43.4%; n = 11/32) rather than younger (30.0%; n = 12/40) women. No significant association between PIK3CA amplification and tumor histologic type, grade, and age of women was observed (Fisher's Exact test: p = 0.660, 0.698 and 0.687, respectively).

Conclusion: PIK3CA gene amplification occurs in about one third of Sudanese women with OC, more frequently in high tumor grades and older women, but not to a statistically significant level. These findings support previous studies suggesting that oncogenic PIK3CA has an essential role in OC progression and may offer a strategy for exact targeted therapy and prognostic evaluation

Keywords

PIK3CA, Gene Amplifications, Ovarian carcinoma, Quantitative Real-Time PCR, Omdurman Maternity Hospital.

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Introduction

Universally, ovarian carcinoma (OC) is the deadliest gynecological tumor. It is ranked as the fifth most prevalent disease causing mortality in women^{1,2}. In 2018, there were 14,070 associated deaths and 22,240 new cases reported in the USA^{3,4}. In Sudan, OC ranked as the fourth most common malignancy in women with 188 in every 100,000 people recorded; 8 in every 100,000 people at a gender-specific rate and 7 in every 100,000 people at an age-standardized rate^{5,6}. In addition, a common issue for OC is that more often a higher stage of carcinoma is seen at diagnosis; resulting in a poorer prognosis for this cancer and the 5-year survival rate of advanced-stage OC remains low^{7,8}.

The phosphatidylinositol-3-kinases (PI3Ks) are a member of the kinase family^{9,10} and have regulatory functions in cell survival, proliferation, and migration^{11,12}. They also have a crucial role in cellular tumorigenesis, cancer development, and drug conflicts^{13,14}. Mechanisms for pathway activation in cancer are mostly due to PIK3Ca or AKT (serine/ threonine-protein kinase B) mutation or amplification^{11,12}. PI3Ks have three classes: class I, class II, and class III^{14,15}; class I is most frequently linked to cancers^{15,16}. Class I is a heterodimer and consists of two main subunits; the regulatory (p85a, p55a, p50a, p85b, p55c, or p101 isoforms) subunit and catalytic (p110a, p110b, p110d, or p110c isoforms) subunit^{16,17}. Among the four catalytic isoforms, only phosphatidylinositol3-kinase, catalytic, α -polypeptide (PIK3CA) gene, which is found on chromosome 3q26.3¹⁸, is frequently altered in cancer^{19,20}.

In OC, PIK3CA gene mutation or amplification is present in >20% of women²¹. To the best of our knowledge, there are no published reports about PIK3CA gene amplification among Sudanese women with OC in Sudan. Therefore, the present study reports the amplification of the PIK3CA gene and its relationship with tumor type, grade, and ages in Sudanese women with OC.

Methods

Clinical samples

This hospital-based, cross-sectional study included 90 adults patients (convenience sampling), out of which, 83 patients diagnosed with OC at Omdurman Maternity Hospital in the period 2013–2018. Another seven healthy ovarian tissues (taken from hospital records of cases diagnosed as unremarkable, which means no histological change) were used as controls to normalize the results. All cases were adults with no history for previous malignancies. The data was collected in the period between 2016 and 2018.

The Ministry of Health of Khartoum state, AlzaiemAlazhari University, and Omdurman Maternity Hospital Ethical Committees approved the study. Informed consent from patients was waived by the committees, since patients' identity was anonymized, and only laboratory numbers were used.

RNA extraction and cDNA preparation

Total RNA was extracted from the FFPE tissue sections using the total RNeasy FFPE kit from Qiagen© (Qiagen, Hilden,

Germany), according to the manufacturer's guidelines. To synthesize cDNA, QuantiTect Reverse Transcription Kit (Qiagen) was used according to the manufacturer's information. The cDNA was stored at -20°C until use.

Quantification of the PIK3CA mRNA

Amplification of the PIK3CA gene was assessed using quantitative real-time PCR (qPCR), performed in ViiA™ 7 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using the QuantiTectSYBR Green PCR Kit (Qiagen). DNA content was standardized to that of long interspersed elements (LINE1), which is a repetitive element with similar copy number per haploid genome, both in normal DNA samples and cancer cells²². The primers for PIK3CA and LINE1 (reference gene as internal control) genes are shown in Table 1.

The whole reaction volume of 20µl consisted of 10µl of SYBR Green Master Mix, 1µl forward and reverse primers (Macrogen, Korea), 1 µl cDNA, and RNA free water to complete the volume to 20µl. The following cycling conditions were used: 95°C for ten mins; 50 cycles with an annealing temperature of 55°C for 30s; and ending with an extension step at 72°C for 30s for both PIK3CA and LINE1 primers. A negative control (nuclease-free water as an alternative of cDNA) was incorporated in each run. A melting curve was obtained to provide evidence for a single reaction product. The relative quantification for PIK3CA gene was calculated using the $2^{-\Delta\Delta C_t}$ method²³. $\Delta\Delta C_t$ represents the difference between the paired tissue samples (ΔC_t tumor - ΔC_t of normal tissue), with ΔC_t being the C_t value for the target gene (PIK3CA) minus the C_t value for the reference gene (LINE1). Values > 2.0 were considered positive for gene amplification.

Statistical analysis

The results were analyzed using SPSS, version 24 (IBM SPSS). Descriptive analysis was performed for all variables (tumor type, grade, and age of women), which were taken from patient records. Fisher's exact test was used between PIK3CA gene amplification and tumor types, grades, and ages for finding statistical significance. P-value < 0.05 was considered to be statistically significant.

Missing data in the dataset (*Underlying data*) concerning PIK3CA gene amplification were considered as undetectable values, which may be due to either a very small expression not detected due to the sensitivity of the method or completely absent, and therefore these samples were included in the analysis as no amplification.

Table 1. Primers for PIK3CA and LINE1 genes used in the present study.

PIK3CA	F, 5'-TATGGTTGTC TGTCATCGGTGA-3'
	R, 5'-GCCTTTGCAGTGAATT-TGCAT-3'
LINE1	F, 5'CCGCTCAACTACATGGAAGCTG-3'
	R, 5'GCGTCCCAGAGATTCTGGTATG-3'

Results

This study included 90 ovarian samples. Among the 90 cases, the median age was 50 years (range, 18–75 years). All cases were distributed into two age groups: ≤ 50 years (range, 18–50 years), 48.2% (n = 40); > 50 years (range, 51–75 years), 38.6% (n = 32).

Amplification of the PIK3CA was common in 33.7% of OC samples (n = 28/83). The results of the amplification rate in different OC types is presented in [Figure 1](#). A higher frequency of amplification was seen in high grade (39.5%; n = 17/43) rather than low grade (27.5%; n = 11/40) carcinoma ([Figure 2](#)). In addition, a higher frequency of amplification was seen in older (43.4%;

Figure 1. The amplification rate of the phosphatidylinositol3-kinase, catalytic, α -polypeptide (PIK3CA) gene in ovarian carcinoma samples according to carcinoma type among Sudanese women.

Figure 2. The amplification rate of the phosphatidylinositol3-kinase, catalytic, α -polypeptide (PIK3CA) gene in ovarian carcinoma samples according to carcinoma grade among Sudanese women.

n = 11/32) rather than younger (30.0%; n = 12/40) women. However, there was no significant association between carcinoma type or grade and age of women, and amplification of PIK3CA (Fisher's Exact test $p = 0.660, 0.698$ and 0.687 , respectively).

Discussion

The phosphatidylinositol-3-kinase (PI3K) pathway plays a regulatory role in common physiological processes; when mutated, it contributes to cellular tumorigenesis, cancer evolution, and drug conflict¹⁴. In addition, it has a decisive role in the progression of OC². Mechanisms for pathway activation are principally due to PIK3CA or AKT mutation or amplification^{11,12}. Earlier studies from developed countries established that amplification was seen in the range of 13 to 24.5% of patients with OC^{24–28}. A single study from a developed country with a small sample size (n=12) reported a higher frequency (40%)²⁹. However, Abubaker *et al.* revealed a high-frequency level (35.5%) in individuals from the Middle Eastern with OC²², which supports the present study. This dissimilarity of the former study with the current study in the frequency may be due to the variation in sample size, ethnic background, the cancer biology in the Sudanese population or the sensitivity of the methods used.

Regarding the association of amplification with tumor grade; we observed an increased amplification frequency in higher grade carcinoma compared with low-grade carcinoma. Our observation implies that PIK3CA gene amplification is an early result in the progress of poorly differentiated (high grade) OC. However, amplification was not significantly associated with tumor type or grade in the present study. Our results are supportive of previous studies^{22,30} where no significant outcome was detected between histological tumor type or grade and PIK3CA amplification.

Limitations

The small sample size limited our study. Further studies with an increased sample size and more additional data (e.g. stage at diagnosis, chemotherapy, radiation) are expected to verify the outcome of this study. The significance of combining other genes, which exist in the PIK/AKT pathway, should also be investigated.

Conclusions

The present study revealed that amplification of the PIK3CA gene occurs in about one-third of Sudanese women with OC, more frequently in high grade carcinoma and women of older ages. In support of recently published literature, our observations foster early reports that imply that oncogenic PIK3CA gene has a significant role in the progression and activation of OC and might offer a new strategy for specific targeted therapy and prognostic assessment in OC patients.

Data availability

Underlying data

Figshare: pik3ca data.csv, <https://doi.org/10.6084/m9.figshare.9121901.v5>³¹

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

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