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ORIGINAL RESEARCH

HIF1α is an independent prognostic factor for overall survival in advanced primary epithelial ovarian cancer – a study of the OVCAD Consortium

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Purpose: Hypoxia is a common phenomenon encountered in solid cancers, leading to chemotherapy resistance and therefore to aggressiveness of the disease. The homeostatic response to hypoxia is mediated by hypoxia-inducible factor-1 (HIF-1). The aim of this study was to investigate the impact of HIF1α in patients with primary epithelial ovarian cancer.

Methods: In this multicentric study, 275 patients with advanced primary epithelial ovarian cancer were included. All patients underwent cytoreductive surgery with maximal surgical effort and adjuvant platinum-based chemotherapy. HIF1α expression was analyzed in tissue lysates, using an enzyme-linked immunosorbent assay.

Results: HIF1α was detected in 79.3% of the tissue samples. Patients with increased HIF1α expression (cutoff: 80 pg/mg protein) in tumoral tissue lysates were more likely to have less favorable survival. HIF1α (P=0.009, hazard ratio [HR] 2.505, 95% confidence interval [95% CI] 1.252–5.013) together with International Federation of Gynecology and Obstetrics (III versus IV) (P=0.013, HR 0.540, 95% CI 0.332–0.878), histology (P=0.007, HR 2.748, 95% CI 1.315–5.743), presence of peritoneal carcinomatosis (P=0.014, HR 2.176, 95% CI 1.170–4.046), residual tumor mass (P=0.017, HR 1.641, 95% CI 1.091–2.468), and response to platinum-based chemotherapy ($P<0.001$, HR 8.131, 95% CI 5.13–12.88) were independent prognosis factors for overall survival. The independent prognostic factors for progression-free survival were International Federation of Gynecology and Obstetrics stage (P=0.01), histological subtypes (P=0.016), and presence of peritoneal carcinomatosis ($P<0.05$).

Conclusion: HIF1α overexpression in ovarian cancer is associated with poor overall survival, underlining the importance of hypoxia in this angiogenesis driven disease.

Keywords: HIF1α, surgical outcome, platinum response, survival, primary epithelial ovarian cancer, predictive factors

Introduction

Ovarian cancer is the leading cause of death from gynecological malignancies, with more than half of patients being diagnosed in the advanced stage. The overall 5-year survival rate is around 40% in International Federation of Gynecology and Obstetrics (FIGO) stages III/IV and 90% in FIGO stage I.[1]

The current standard of treatment for primary ovarian cancer is the combination of optimal cytoreductive surgery and platinum-based chemotherapy. Despite state of the art treatment, more than 50% of the patients will relapse and consequently die from this disease.[2] A curative approach is only possible in the primary situation. The aim of targeted

therapy is to prolong the tumor-free status achieved after the primary treatment. Recent data showed that some antiangiogenic therapies, eg, bevacizumab, are able to prolong progression-free survival, thus no improvement in overall survival has been shown yet.[3,4] There is an urgent need to identify new targets, and also biomarkers able to predict platinum response.

No macroscopic residual tumor mass after cytoreductive surgery is one of the major prognostic and predictive factors. Despite radical surgery, maximal tumor reduction cannot always be achieved. A neoadjuvant approach could have a benefit in this subgroup of patients, although data are controversial.[5–8] Nevertheless, there are no effective biomarkers to predict surgical outcome in primary ovarian cancer patients.

HIF1α has been reported to be an important predictor of tumor progression for several types of solid cancers.[9–13] HIF1α is a major regulator of cell adaptation to hypoxic stress and plays a critical role in oncogenesis and angiogenesis.[14] This protein regulates the transcription of a number of genes involved in diverse biological functions such as proliferation, migration, invasion, apoptosis, and angiogenesis.

Immunohistochemical analysis of human cancer biopsies has shown that HIF1α is overexpressed in several solid malignancies, including breast, colon, lung, gastric, skin, and renal carcinomas, compared with their respective normal tissues.[15,16]

Like other tumors, overexpression of HIF1α has been detected in epithelial ovarian cancer.[15] Data regarding the clinical impact of HIF1α in ovarian cancer is still limited. Although HIF1α is detected in all epithelial ovarian cancer histological subtypes, higher HIF1α expression in clear-cell carcinomas has been reported.[17,18]

The role of HIF1α in the development of new targeted therapies has been suggested by recent studies.[19,20] Nakai et al stated that HIF1α-expressing tumors had a significantly higher response rate to postoperative platinum-based chemotherapy than tumors without HIF1α expression.[21]

However, the prognostic significance of HIF1α overexpression in ovarian cancer is still controversial.[21–24] Until now, only limited data exist regarding the HIF1α predictive value in epithelial ovarian cancer.

The aim of this study was to investigate the impact of HIF1α expression in predicting surgical outcome, platinum response, and survival in patients with advanced primary epithelial ovarian cancer.

Patients and methods

This analysis was performed within the Sixth Framework Program European project "Ovarian Cancer – Diagnosis of a Silent Killer" (OVCAD). At five comprehensive centers for ovarian cancer treatment, 275 patients with advanced primary epithelial ovarian cancer (FIGO stage II–IV) were enrolled.

All patients gave their written informed consent before tissue samples were collected. Patients were recruited from 2005–2008 in the Department of Gynecology at Charité – Medical University Berlin (Berlin, Germany), Department of Gynecologic Oncology, University Hospital Leuven (Leuven, Belgium), Department of Obstetrics and Gynecology, Medical University of Vienna (Vienna, Austria), Department of Gynecology, University Medical Center Hamburg-Eppendorf (Hamburg, Germany), and Department of Gynecology and Obstetrics, Innsbruck Medical University (Innsbruck, Austria). This study was approved by each local ethics committee (EK207/2003, ML2524, HEK190504, EK366, and EK260).

Only patients with histologically confirmed ovarian cancer were included in the study. Due to better prognosis and sometimes different therapy strategies, FIGO stage I patients were excluded. All patients underwent cytoreductive surgery with maximal surgical effort and platinum-based chemotherapy. Inclusion and exclusion criteria for the study were reported in a recent publication.[25] An online database was used for the documentation of clinical, histopathological, and follow-up data. Residual tumor load was defined as negative if macroscopically absent. Overall survival was defined as the time from diagnosis of ovarian cancer to tumor-related death or last contact. Progression-free survival was defined as the interval from diagnosis to progression of disease or death, whereby progression was defined as an increase in the nadir serum CA-125 level of at least two samples according to the Gynecological Cancer Intergroup criteria or radiological diagnosis according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria.

Documentation of clinical data

The tumor spread within the abdominal cavity was documented prospectively using the Intraoperative Mapping of Ovarian cancer – "IMO" tool.[26] The documentation of residual tumor mass was also assessed prospectively at the end of the surgery with an interview with the main surgeon. All data were documented in a validated online database.

The FIGO 2006 classification was used for clinical staging, and the Gynecologic Oncology Group criteria were used for histologic grading.[27] Response to treatment and diagnosis of recurrence was determined according to RECIST criteria

or according to CA-125 variations (Gynecological Cancer Intergroup criteria) during follow-up.[27,28]

Response to first-line chemotherapy was defined according to the last time patients received platinum-based compounds. Patients developing relapse within the first 6 months after platinum based chemotherapy were classified as platinum-resistant; patients developing relapse after 6 months or more were classified as responders.[29]

Collection of tumor tissue

Tumor tissue was removed from the patients during surgery and prior to chemotherapy treatment. Samples were snap frozen and stored in liquid nitrogen. At a later time, the tissue was processed to obtain lysate for protein measurement.

About 30 mg tumor tissue was homogenized with a Mikro-Dismembrator and lysed in 1 mL Nucleic Acid Purification Lysis Solution (Thermo Fisher Scientific, Waltham, MA, USA).

HIF1α enzyme-linked immunosorbent assay (ELISA)

HIF1α expression in tissue lysates was measured using the HIF1α ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer's protocol. The assay was performed in duplicate for the calibrators, controls, and patient samples. The appropriate controls were within the ranges provided by the manufacturer.

Statistical analysis

The clinical data were collected and entered in an online database. The statistical analysis was performed using IBM® SPSS® Statistics 21.0 (IBM Corporation, Armonk, NY, USA) at Charité – Medical University Berlin.

The prognostic factors used in the survival analysis were as follows: age at first diagnosis, FIGO stage, histological subtype, histological grade, presence and volume of ascites, residual tumor mass after surgery, peritoneal dissemination, and responses to platinum-based chemotherapy.

Kruskal–Wallis test, Spearman's rank correlation, Kendall's tau, and Mann–Whitney *U* test were used to assess the associations between HIF1α and other variables.

Receiver operating characteristic curve analysis was performed to evaluate the predictive accuracy of HIF1α expression for discriminating patients with maximal versus suboptimal residual tumor mass after surgery and platinum responders versus nonresponders.

Median survival and 95% confidence intervals (95% CI) were estimated according to the Kaplan–Meier method. The log-rank tests were used for univariate statistical comparisons and the Cox proportional hazard model was used to evaluate significant predictors of survival. Adjusted hazard ratios (HR) and 95% CI for prognostic factors were calculated. A two-tailed P-value <0.05 was considered statistically significant.

Results

Baseline characteristics

Most of the patients (86.2%) were diagnosed with serous ovarian cancer. Optimal debulking, ie, no evidence of macroscopic tumor residuals, was reported in more than 60% of the patients. Patients' characteristics are presented in Table 1, and are reported in greater detail elsewhere.[25]

Table 1 Patient's clinical and pathologic characteristics

Parameter	Primary ovary cancer
Ovarian cancer samples included to the multicenter study, n (%)	275 (100.0)
Age at time of diagnosis, median (range), years	58 (18–85)
Histological type, n (%)	
Serous	237 (86.2)
Endometrioid	13 (4.7)
Mixed/others*	25 (9.1)
Tumor stage, n (%)	
FIGO II	15 (5.4)
FIGO III	212 (77.1)
FIGO IV	48 (17.5)
Lymph node status, n (%)	
N0	65 (23.6)
N1	143 (52)
Nx	67 (24.4)
Distant metastatic spread, n (%)	
M0	142 (51.6)
M1	49 (17.8)
Mx	84 (30.5)
Grading, n (%)	
Well differentiated	10 (3.6)
Moderately differentiated	64 (23.3)
Poorly differentiated	200 (72.7)
Unknown	1 (0.4)
CA-125 level, n (%)	258 (93.8)
Preoperatively, median (range), U/mL	1,500.24 (7–37,820)
Ascites volume, n (%)	
None	66 (24)
≤500 mL	110 (40)
>500 mL	100 (36)
Peritoneal carcinomatosis, n (%)	
Yes	186 (67.6)
No	89 (32.4)
Postoperative residual tumor mass, n (%)	
Macroscopic tumor free	188 (68.4)
≤1 cm	48 (17.5)
>1 cm	38 (13.8)
Missing	1 (0.4)

Note: *Eleven mixed, nine undifferentiated, and two clear-cell carcinomas.
Abbreviation: FIGO, International Federation of Gynecology and Obstetrics.

Expression of HIF1α

Expression of HIF1α was confirmed in 218 (79.3%) patients. No expression of HIF1α was observed in 57 tissue samples (20.7%). The median HIF1α expression was 56.8 (range 0–1,492.31) pg/mg protein.

Correlation with classical prognostic factors

No statistically significant association between HIF1α expression and clinical prognostic factors such as age (P=0.238), volume of ascites (P=0.866), FIGO stage (P=0.0896), and grading (P=0.152) was found.

Levels of HIF1α were higher in patients presenting peritoneal carcinomatosis and mucinous and mixed histology, although this correlation retained no statistical significance (P=0.065 and P=0.071, respectively).

There was no correlation between HIF1α and residual tumor mass after cytoreductive surgery (P=0.342, area under the curve 0.555, 95% CI 0.471–0.639) or response to platinum-based chemotherapy (P=1, area under the curve 0.499, 95% CI 0.413–0.586) (Table 2).

Survival analysis

The median follow-up time for patients was 37 months, ranging from 1–69 months; 194 patients developed recurrent disease and 134 patients died from ovarian cancer.

In order to analyze the role of HIF1α for survival, the lower and upper quintiles were used as cutoff values. Using the cutoff values of 15 pg/mg protein and 80 pg/mg protein, it could be shown that patients presenting HIF1α tissue concentrations higher than 80 pg/mg protein would have a shorter overall survival, although this association was not significant within the univariate analysis. When multivariate analysis was performed, HIF1α concentrations over 80 pg/mg protein remained an independent prognostic biomarker for overall survival (P=0.009, HR 2.505, 95% CI 1.252–5.013) (Figure 1) together with FIGO (III versus IV) (P=0.013, HR 0.540, 95% CI 0.332–0.878), histology (mixed versus serous versus endometrioid) (P=0.007, HR 2.748, 95% CI 1.315–5.743), presence of peritoneal carcinomatosis (P=0.014, HR 2.176, 95% CI 1.170–4.046), residual tumor mass (present versus absent macroscopically) (P=0.017, HR 1.641, 95% CI 1.091–2.468), and response to platinum-based chemotherapy (P<0.001, HR 8.131, 95% CI 5.13–12.88).

FIGO stage (P=0.001, HR 0.478, 95% CI 0.314–0.726), presence of peritoneal carcinomatosis (P<0.001, HR 2.620, 95% CI 1.704–4.027), and histology (P=0.021, HR 2.306, 95% CI 1.137–4.677) were the only independent predictive factors for progression-free survival.

Table 2 Correlation with clinical prognostic factors

Clinical parameters	HIF1α median (pg/mg protein)	*P*-value
FIGO stage		
FIGO stage II	36.9	0.896
FIGO stage III	35.7	
FIGO stage IV	34.6	
Histology		
Serous	34.8	0.071
Endometrioid	33.4	
Mixed	77.1	
Mucinous	19.9	
Grading		
G1	53.4	0.152
G2–G3	35.1	
Platinum response		
Responder	35.1	1
Nonresponder	35.7	
Peritoneal carcinomatosis		
Present	38	0.065
Absent	30	
Ascites		
Absent	34.5	0.914
≤500 mL	35.1	
>500 mL	37.1	
Residual tumor mass		
Absent	33.3	0.342
Present	41.5	

Abbreviation: FIGO, International Federation of Gynecology and Obstetrics.

Discussion

This study analyzed the role of HIF1α in primary ovarian cancer. The results showed that patients having tumors that overexpress HIF1α transcription factor (cutoff 80 pg/mg protein) are presenting a poorer prognosis.

One of the major bottlenecks in ovarian cancer management is the development of platinum resistance with

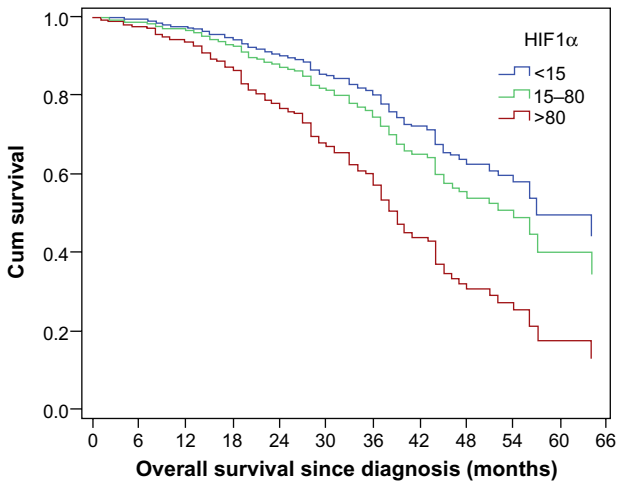

Figure 1 HIF1α expression and overall survival (P=0.009, hazard ratio 2.505, 95% confidence interval 1.252–5.013).

subsequently increased mortality. The development of new targeted, especially antiangiogenic, drugs has translated into prolonged progression-free survival without evidence of a positive impact on overall survival. The inhibition of neoangiogenesis in ovarian cancer leads to a certain hypoxia of the tumoral cells, and therefore increases apoptosis of tumoral cells. There are no in sufficient data to show a benefit in overall survival for patients being treated with antiangiogenic drugs. This might be caused by the capability of tumor cells to resist even hypoxic conditions.

One of the mechanisms of chemotherapy resistance in solid tumors is the ability to develop a microenvironment caused by a poor and inefficient vascular supply.[30] The presence of hypoxia has been shown to be a marker for poor prognosis in many cancer types. The resistance to conventional chemotherapeutical drugs is caused by hypoxia-induced reduction in cell proliferation and also by the fact that hypoxic tumor cells reside some distance from the blood vessels, and therefore the drugs won't be able to reach their target.[31,32] The homeostatic response to hypoxia is mediated by the transcription factor HIF1α, which is unstable in well-oxygenated tissue but becomes stable under hypoxic conditions.[33] Due to hypoxic conditions in tumoral tissue, as described previously, HIF1α is an important mediator for tumor invasion, metastasis, and chemotherapy resistance.[34]

This study showed that HIF1α expression was independent of FIGO stage, age, and grading. Mucinous and mixed epithelial carcinomas showed increased levels of HIF1α, although this association didn't reach statistical significance. Previously published data showed that clear-cell carcinomas exhibit higher HIF1α levels than serous, mucinous, or endometrioid tumors.[35,36] Other studies suggest that HIF1α might be overexpressed in serous ovarian cancer.[24] The difference in the current results could be caused by low numbers of well-differentiated (3.6%) and non-serous ovarian cancer patients included within the OVCAD study. More than that, differences in ethnicity might cause differences in the molecular biology of ovarian cancer. It is well known that clear-cell ovarian cancer is more often encountered in Asian women compared to Caucasian women, and the clinical course of the disease is different. Clinical studies showed that chemotherapy or targeted therapy drugs are associated with different response rates and adverse effects in Caucasian and Asian populations.[37]

Results similar to the current study were published by Nakayama et al, who reported that HIF1α levels were independent of clinical stage, age and histological subtypes.[38] HIF1α expression was reported to be significantly stronger in good differentiated ovarian cancer.[17] The current results showed no significant correlation between HIF1α tissue expression and histological grading, which might be explained by the low number of well-differentiated (G1) ovarian cancers included in this study. There was also no significant influence of HIF1α expression on response to platinum-based chemotherapy, this being in accordance with some existing results.[17] However, Nakai et al reported that tumors expressing higher HIF1α more likely respond to platinum-based chemotherapy compared with tumors with no or poor HIF1α expression.[21] The current study analyzed the expression of HIF1α in 275 patients with FIGO stage II–IV, but Nakai et al's study only included 52 stage III–IV epithelial ovarian cancer patients.

HIF1α increased tissue values were associated with shorter progression-free survival, but this was not statistically significant, which agrees with recent studies.[22,23] Daponte et al observed that the median progression-free survival of HIF1α-positive patients was shorter, but not statistically significant.[22] In lung and colorectal carcinomas, HIF1α had no impact on patient survival, but overexpression of HIF2α was a prognostic indicator.[39,40] In ovarian carcinomas, Birner et al showed that HIF1α overexpression alone was not a prognostic indicator and became a strong prognostic marker in combination with functional p53 protein.[17]

Conclusion

The current study showed that HIF1α tissue expression might impact the overall survival in primary ovarian cancer patients treated by cytoreductive surgery and platinum-based chemotherapy. No correlation between HIF1α tissue expression and platinum response and no significant statistical difference between HIF1α expression in different histological subtypes was found. Further studies analyzing the role of this transcriptional factor in a larger prospective cohort of patients are warranted. Studies analyzing the HIF1α distribution within tumor tissue using immunochemistry are needed.

Drugs interfering with the HIF1α pathway, such as ganetespib,[41] are currently under evaluation in clinical studies. Targeting HIF1α might be a way to improve survival in ovarian cancer patients.

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Disclosure

The authors report no conflicts of interest in this work.

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